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Infant TB Infection Prevention Study (iTIPS): a randomized trial evaluating isoniazid to prevent M. tuberculosis infection in HIV-exposed uninfected children

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Infant TB Infection Prevention Study (iTIPS): a randomized trial evaluating isoniazid to prevent *M. tuberculosis* infection in HIV-exposed uninfected children

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ABSTRACT

Introduction

HIV-exposed uninfected infants (HEU) in TB endemic settings are at high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in the absence of known Mtb exposure. Because infancy is a time of rapid progression from primary infection to active TB disease, it is important to define when and how TB preventive interventions exert their effect in order to develop effective prevention strategies in this high-risk population.

Methods and Analysis

We designed a non-blinded randomized controlled trial to determine efficacy of isoniazid (INH) to prevent primary Mtb infection among HEU children. Target sample size is 300 (150 infants in each arm). Children are enrolled at 6 weeks of age from maternal and child health clinics in Kenya and are randomized to receive 12 months of daily INH ~10 mg/kg plus pyridoxine or no INH. The primary endpoint is Mtb infection, assessed by interferon-gamma release assay (IGRA) QuantiFERON-TB Gold Plus (QFT-Plus) or tuberculin skin test (TST) after 12 months post-enrollment. Secondary outcomes include severe adverse events, expanded Mtb infection definition using additional QFT-Plus markers, and determining correlates of Mtb infection. Exploratory analyses include a combined outcome of TB infection, disease, and mortality, and sensitivity analyses excluding infants with baseline TB-specific responses on flow cytometry.

Ethics and Dissemination

The protocol is approved by the ethical review boards of University of Nairobi/Kenyatta National Hospital, Jaramogi Oginga Odinga Teaching and Referral Hospital, and University of Washington, and Kenya Pharmacy and Poisons Board. An external and independent Data and Safety Monitoring Board monitors adverse events. Results will be disseminated through peer-reviewed journals, presentations at local and international conferences to national and global policy makers, the local community, and participants.

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Keywords: pediatric, tuberculosis, prevention, isoniazid, protocol

ARTICLE SUMMARY

Strengths and limitations of this study

- With successful prevention of maternal-to-child HIV transmission programs, more children born to mothers with HIV are HIV-exposed but uninfected (HEU), but may remain at high risk of TB due to household and community exposure in areas of high HIV/TB burden, making them an important population in which to study TB prevention.

- Current TB prevention guidelines do not recommend routine isoniazid preventive therapy (IPT) for children <12 months of age with HIV due to conflicting data regarding IPT effectiveness at this age, and gaps remain regarding the role of IPT in HEU children without a known TB exposure.

- A prospective HEU cohort using both interferon-gamma release assays (IGRA) and tuberculin skin tests (TST) to detect *M. tuberculosis* (Mtb) infection can provide an efficient approach to probe determinants of Mtb infection, more rapidly accruing endpoints (Mtb infection) than studies of TB disease, and can contribute unique insights regarding prevention of primary Mtb infection strategies.

- Given equipoise regarding whether INH prevents Mtb infection in general, and a lack of data specifically among HEU children, an RCT design could provide important information regarding the efficacy of INH in preventing Mtb infection in this high-risk population.

INTRODUCTION

HIV-exposed uninfected infants (HEU) in tuberculosis (TB) endemic settings have a high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in the absence of known Mtb exposure.[1, 2] Because infancy is a time of rapid progression from primary infection to active TB[3], it is important to know how TB preventive interventions exert their effect to build new strategies that adapt or extend approaches used in adults. Protecting HEU infants during this vulnerable period of immunodeficiency may provide long term benefits.

Children have a higher rate of progression from infection to active disease than adults.[4, 5] In young children who lack pre-existing adaptive immune responses, Mtb infection progresses rapidly to TB disease, and both innate and early adaptive immune responses may influence susceptibility.[4, 6-8] Virtually all childhood TB disease reflects primary disease, in contrast to adults where a significant portion of disease is due to reactivation of latent TB infection.[9] The World Health Organization (WHO) recommends IPT be provided to people living with HIV (PLHIV) >12 months of age.[10] Among children living with HIV (CLHIV), three randomized controlled trials (RCTs) yielded conflicting data regarding whether isoniazid (INH) prevents TB disease and/or mortality.[11-14] Only one evaluated HEU infants, and found no protective effect of INH in decreasing TB disease.[11] While previous RCTs have focused on prevention of active TB disease, there are scant data regarding impact of INH on primary Mtb infection. Among 6-month old HEU infants in Kenya, we found that over 10% had evidence of Mtb infection as detected by interferon-gamma release assays (IGRAs), suggesting a potential 20% annual cumulative incidence of Mtb infection.[15] Among children in Uganda (median age 36 months), prevalence of Mtb infection (either TST $\geq$10 mm or positive IGRA) was 2-fold higher among HEU compared to HIV-unexposed children (HUU), with higher prevalence of TST-positivity vs. IGRA in both groups (HEU TST 27.2% vs. IGRA 6.4%, HUU TST 20.6% vs. 1.5%).[16] This suggests that HEU infants have a substantial incidence of Mtb infection,[17, 18] as well as low to modest concordance between different Mtb infection measures.

There is currently no gold standard for Mtb infection diagnosis.[19] While TST is recommended for detection of Mtb infection in children under 5 years of age,[20, 21] false positivity due to Bacille Calmette-Guérin (BCG) vaccination can occur.[22] Data regarding performance of IGRAs in young children is scarce, however a recent study of BCG-immunized infants in South Africa indicate high IGRA Quantiferon IFN-γ conversion values were strongly associated with subsequent development of active TB.[23] Because IGRAs offer increased specificity for Mtb infection detection in the presence of recent BCG vaccine, it is plausible that IGRAs could enhance the ability to measure preventive effect of INH.[22] IGRA and TST agreement in children varies widely and appears affected by nutrition and HIV status, TB burden, and BCG immunization.[24-28] Recent ATS/IDSA/CDC guidelines recommend dual testing IGRA and TST for groups who are both likely to be infected and at high risk of progression to TB disease as a strategy to increase diagnostic sensitivity.[21] Reduction of specificity with this strategy may be acceptable when consequences of missed Mtb infection (and therefore missed opportunity for treatment) outweigh risks of therapy-associated adverse events. Few longitudinal studies of Mtb infection among young infants including HEUs using serial IGRA and TST testing.[23, 29] A prospective infant HEU cohort using both IGRA and TST to detect Mtb infection can provide an efficient approach to probe determinants of Mtb infection, more rapidly accruing endpoints (Mtb infection) than studies of TB disease. This study design can contribute unique insights regarding Mtb infection prevention strategies.

Current Kenyan guidelines mirror WHO guidelines and recommend IPT for all known TB-exposed children <5 years of age and for all HIV-infected children >1 year of age regardless of TB-exposure.[10, 30] However for children <5yr who are not TB-exposed (including HEU), and for HIV-infected children <1 year, IPT is not recommended.[10, 30] These guidelines illustrate uncertainty regarding IPT in young children, following an RCT from South Africa/Botswana that failed to demonstrate IPT effectiveness in preventing TB disease among CLHIV and HEU <1 year of age without known TB exposure.[11-14] However, it remains possible that among HEU children exposed to unperceived community or household TB, INH may prevent primary

Mtb infection. Although data are conflicting, some adult studies have demonstrated IPT benefit in TST- or IGRA-negative adult PLHIV suggesting IPT may confer protection from Mtb infection.[31, 32]

The primary goal of this study is to determine whether INH prevents primary Mtb infection in HEU infants, to determine timing and cofactors of primary Mtb acquisition in the first year of life, and to examine the role of immune protective mechanisms in this cohort (Figure 1). This paper details the study protocol of an RCT evaluating efficacy of a 12-month course of INH to prevent Mtb infection as measured by IGRA and/or TST in HEU children enrolled at 6 weeks of age in western Kenya.

METHODS AND ANALYSIS

Study Design

The infant TB Infection Prevention Study ("iTIPS") is a 2-arm, non-blinded RCT comparing efficacy of a 12-month course of daily INH vs. no INH to prevent Mtb infection among HIV-exposed uninfected Kenyan children enrolled at 6 weeks of age (Figure 2). Eligible infants are randomized using a 1:1 allocation to INH vs. no INH (Figure 3).

Study sites

Kenya is one of 22 high TB burden countries with a generalized TB epidemic[33], with an estimated TB prevalence of 426 per 100,000.[34] This study is conducted in collaborative research sites in western Kenya embedded in Ministry of Health (MOH) maternal child health (MCH) clinics. HIV-infected mothers in Kenya are followed as part of the national prevention of maternal to child transmission (PMTCT) program and currently receive Option B+ triple antiretroviral therapy.[35] Per Kenyan guidelines, all PLHIV should be screened at routine HIV care visits using symptom-based TB screening and those with negative screens are evaluated for IPT.[10, 33, 35] Current WHO and Kenyan guidelines do not specifically recommend IPT for HEU infants without known TB contact.

Recruitment processes & eligibility criteria

We recruit HIV-infected mothers and their HEU infants from MCH/PMTCT sites 6-10 weeks after birth. Infants are recruited during routine 6-week immunization visits, but a 4-week window is allowed for infants presenting late for this visit.

Infants aged 6 weeks (+ 4 weeks) of age are eligible for inclusion if they are born to HIV-infected mothers, with birthweight ≥2.5 kilograms, and not born premature (<37 weeks gestation). Infants with known household TB exposure are ineligible. Infants enrolled in other TB prevention or TB vaccine studies are ineligible because these interventions might affect ascertainment of endpoints.

Randomization

Site-stratified randomization is used to allocate infants 1:1 to INH or no INH arms. Randomization numbers were generated prior to study start by a study biostatistician using STATA 14 "*ralloc*" command with resulting randomization assignment by participant ID printed on cards and placed in opaque envelopes.

Blinding

The study is non-blinded to enable prompt clinical management for any potential drug-related adverse event. IGRAs are performed in the Kenya Medical Research Institute (KEMRI) Centers for Disease Control (CDC) laboratory, which is blinded to arm. The study team administers TST and not blinded to TST result. Data monitoring by the study team is not disaggregated by study arm. Only the study biostatistician reviews data by arm during preparation of reports to the external Data and Safety Monitoring Board (DSMB). This data is reviewed during closed DSMB sessions, which excludes team members involved in study implementation.

Enrollment and Study procedures

Enrollment

After informed consent is obtained by study staff, household locator information, medical identification number, and cellphone contacts are obtained to facilitate tracing. On enrollment, standardized questionnaires regarding sociodemographic, clinical, obstetric and HIV-related factors, TB exposure and

history, and TB symptoms (for infant, mother, and household members using WHO symptom screen[36]) are administered (Supplemental Table 1). Mothers with suspected TB are referred to the TB program for further screening. If mothers are found to have TB on enrollment, their infants are ineligible for participation and are referred to receive INH per Kenya national guidelines.

Infants undergo physical examination measuring weight, height/length, mid-upper arm circumference, and presence of Bacillus Calmette-Guerin (BCG) scar. Medical records are used to abstract data on infant birthweight, PMTCT prophylaxis, other medications, immunizations, and maternal ART regimen, viral load and CD4 cell counts.

Intervention

Isoniazid ~10 mg/kg (7-15 mg/kg) is administered once daily to infants in the INH arm for 12 months. Standardized weight-based dosing (by weight band using 100 mg scored tablets) is used, corresponding to Kenya and WHO recommendations.[30, 37] Pyridoxine is provided to children randomized to INH to decrease peripheral neuropathy risk.[30, 37] Caregivers are advised on how to pulverize isoniazid and pyridoxine to be mixed with small quantities of breastmilk, clean water, or liquid co-trimoxazole to ensure full doses are given and for ease of administration to infants. Participants in the intervention arm are administered daily INH and pyridoxine by caregivers. Infants in the control arm do not receive INH or pyridoxine.

Participant follow-up

Follow-up visits occur at 10 and 14 weeks, and 6, 9, and 12 months of age coinciding with routine Kenya pediatric visit schedule. Questionnaires regarding caregiver barriers and facilitators to providing prophylactic medications (co-trimoxazole, ART for PMTCT, and IPT [if in IPT arm]) to infants are administered at 6 month of age visit. Endpoint ascertainment occurs at a study visit 12 months post-randomization at approximately 14 months of age.

Sample collection

Infant blood for peripheral blood mononuclear cells (PBMC) and plasma are collected at baseline and visit 2 (10-14 weeks of age). At 12 months post-randomization, blood is collected for IGRA (QFT-Plus) and TST placed and read within 48-92 hours.[38, 39] Infant rectal swabs are collected at enrollment for future gut microbiome studies. Maternal breastmilk and blood for PBMC and plasma separation are collected on enrollment.

Study procedures specific to infants randomized to INH

For infants randomized to INH, liver function tests (LFTs) are performed at enrollment and one month following INH initiation. Adherence is assessed by caregiver report at follow-up visits. Urine is collected at follow-up and study endpoint visits and tested using strips developed to detect INH-metabolites.[40, 41] Hair is collected at study endpoint for future assessment of isoniazid levels as a more objective adherence measure over time.[42, 43]

Safety considerations

IPT has been shown to be safe in prior RCTs and is administered routinely to TB-exposed infants.[11-14] Although routine LFT monitoring is not recommended during INH in children,[20] for this trial baseline LFTs are drawn at enrollment and one month after INH initiation for infants randomized to INH. NIH Division of AIDS (DAIDS) Table for Grading the Severity of Pediatric Adverse Events is used to grade toxicities.[44] Infants with LFTs $\geq$ grade 3 at baseline have LFTs monitored every 2 weeks and do not initiate INH until LFTs are $\leq$ grade 2. After INH initiation, if toxicity is suspected, study administered drugs are immediately discontinued and in the case of concern for hepatoxicity, LFTs are repeated. If LFTs are $\geq$ grade 3, we follow the participant until resolution of the toxicity to $\leq$ grade 2. For $\geq$ grade 3 hepatic abnormality supported by repeat laboratory tests, we hold INH for two weeks and recheck LFTs. If a $\geq$ grade 3 abnormality is still present after withholding INH for two weeks, we continue to hold INH and recheck LFTs in 2 weeks.

An external and independent DSMB, including experts in pediatric TB, biostatistics, and trial design, monitors severe adverse events (SAE). Summaries of SAEs are given to DSMB members during scheduled meetings. Each SAE is assigned the plausibility of relatedness to study drug by study investigators. "Open" reports detailing cumulative overall SAEs are descriptive (no statistical analyses). "Closed" reports of SAE data by study arm are also reviewed and the DSMB makes recommendations regarding any imbalances in safety outcomes. O'Brien-Fleming boundaries for benefit and harm are used for interim monitoring and these boundaries are provided by the study statistician in the closed reports. The DSMB reviews the interim reports and assess the study in terms of operational aspects, safety, and effectiveness and makes recommendations regarding study continuation or modifications. Futility is not considered a basis for stopping rules because of the trials' value in understanding epidemiologic and immunologic correlates of Mtb infection in HEU infants.

Discontinuation, withdrawal, or allocation modification

Participants may withdraw consent for participation at any point. Study investigators may withdraw a participant from the study on a case-by-case basis if the intervention or study involvement poses a risk to the participant. No modification of allocation will be made. Infants who receive at least one dose of study drug will be included in primary outcome per protocol analyses even in the setting of intervention discontinuation or withdrawal from study. Caregivers of infants who discontinue INH are encouraged to continue study follow-up and endpoint ascertainment.

Data collection and management

Study staff use tablets to collect de-identified data using secure password-protected Research Electronic Data Capture mobile software (REDCap, Vanderbilt University, Nashville, Tennessee, USA).[45] Data are uploaded daily from tablets to the web-based REDCap database. Study investigators will have access to the finalized dataset.

Outcome measures

The primary outcome is the proportion of participants with Mtb infection defined as either a positive QFT-Plus assay or TST 12 months after enrollment. Similar to QuantiFERON-TB Gold (QFT) IGRA, QFT-Plus measures IFN-γ released by primarily CD4+ T helper lymphocytes after TB-specific antigen (ESAT-6 and CFP-10) stimulation. In addition, QFT-Plus measures IFN-γ released by CD8+ cytotoxic T lymphocytes, after stimulation with the same antigens, which may have increased sensitivity in children, and in populations with lower CD4 counts including HIV.[46, 47] A response of ≥0.35 IU/ml to TB antigens above the Nil response in either the primarily CD4+ response [TB1], or CD8+ response [TB2] (with Nil <8 IU/ml and a positive mitogen control) are considered a positive result per manufacture recommendations.[46] A TST of ≥10 mm is considered positive.[20]

Secondary outcomes include severe adverse events (Grade ≥3 per DAIDS Grading Severity of Pediatric Adverse Experiences),[44] use of IFN-γ-independent immune markers in QFT-Plus supernatants to indicate Mtb infection,[48-52] and epidemiologic and immunologic correlates of Mtb infection. Exploratory outcomes include combined endpoint of Mtb infection, TB diagnosis, and/or death, as well as sensitivity analyses of the primary outcome of Mtb infection after excluding infants with evidence of immune responses to ESAT-6 or CFP-10 at enrollment in flow cytometric analyses.

Sample size and power analysis

The primary endpoint is Mtb infection (by TST or QFT-Plus). Assuming alpha of 0.05, power of 0.80, utilizing a 2-sided test, and a 1:1 allocation ratio, with 125 infants in each arm we have power to detect at least 65% decrease in Mtb infection in INH arm vs. control if cumulative incidence of Mtb infection in control arm at 12 months follow-up is 0.2, or to detect 70-80% or higher (HR 0.3-0.2) decrease if cumulative incidence of Mtb infection in control arm is 0.15 or 0.1 (Supplemental Table 2). To account for loss to follow-up, non-adherence, and isoniazid resistance, we increased sample size by 20%, with goal enrollment of 300 infants (150 per arm).

Statistical methods and analysis

Primary outcome:

Modified Intention-to-treat: The primary modified intention-to-treat analysis will use a Chi-squared test to compare proportion of participants with Mtb infection (by either QFT-Plus or TST) at 12 months following enrollment between INH and no INH arms, excluding infants found to be HIV DNA positive at enrollment or during the study. Baseline characteristics will be compared between randomization arms to assess randomization adequacy.

Per protocol: We will also evaluate our primary outcome by a per protocol analysis, considering only HEU infants who took at least one dose as taking INH vs. infants who did not take any INH. We anticipate future sensitivity analyses using IPT adherence and continuation data as exposure of interest and Mtb infection as outcome.

Secondary outcome:

Safety and expanded Mtb infection outcomes: For secondary outcomes we will compare proportions of participants by arm using either Chi-squared or Fisher's exact tests as appropriate for ≥ grade 3 serious adverse events considering only children who received at least one dose of INH in the INH arm. In addition, we will conduct secondary analyses using an expanded Mtb infection definition including a positive TST, QFT-Plus, or IFN-γ-independent immune markers in QFT-Plus supernatants.

Epidemiologic and immune correlates of Mtb infection will be assessed using nested case-control studies incorporating all Mtb infections from both arms and then conducting stratified analyses in each trial arm to evaluate potential cofactors modified by INH.

Exploratory outcome:

We will compare composite endpoint of Mtb infection, TB diagnosis, and/or death between randomization groups using a Chi-squared test. Baseline assays may detect evidence of Mtb infection. We will conduct an additional exploratory analysis, incorporating data from baseline assays[53] (utilizing flow

cytometry of cryopreserved PBMCs) to exclude infants with evidence of immune responses to ESAT-6 or CFP-10 at enrollment also using a Chi-squared test.

ETHICS AND DISSEMINATION

Ethical considerations

Informed consent is obtained from caregivers. The trial protocol is approved by ethical review boards of University of Washington, University of Nairobi/Kenyatta National Hospital, and Jaramogi Oginga Odinga Teaching and Referral Hospital, and Kenya Pharmacy and Poisons Board. The study is registered at clinicaltrials.gov (NCT02613169). Any protocol changes will be approved by relevant ethical review boards and clinicaltrials.gov will be updated.

HEU children are at increased risk for Mtb infection and TB disease. IPT is not routinely provided to HEU infants in Kenya without evidence of exposure to a known TB case. There is mixed evidence regarding IPT effectiveness to prevent TB disease in infants <1 year. Given potential benefits of IPT to prevent Mtb infection, heightened risk for Mtb infection in this population, and safety of intervention, there is equipoise for randomization.

Trial status

Trial recruitment and enrollment began August 15, 2016. Participant follow-up is anticipated to complete September 2019, with lab analyses anticipated to be completed in December 2019.

Dissemination plans

We will share trial results at study sites, and with regional and national policymakers. We plan on submitting final results as a peer-reviewed manuscript and will utilize International Committee of Medical Journal Editors authorship criteria. Study investigators will collaborate in writing final study results.

DISCUSSION

Isoniazid has proven benefit to treat latent TB infection and prevent active TB disease in HIV-infected and HIV-uninfected populations.[54-58] Data from adult studies in Botswana and South Africa indirectly

suggest IPT may prevent Mtb infection; TST negative adult PLHIV who received IPT were protected from active TB, suggesting IPT may both prevent Mtb infection and progression to TB disease.[31, 32] IPT has had variable protective efficacy to prevent TB disease and mortality in CLHIV.[11-14] An RCT in South Africa in the pre-ART era randomized CLHIV ≥8 weeks of age to INH vs. placebo (independent of reported TB exposure) and found INH prevented TB disease by 70% and decreased mortality by 54%, leading to early trial discontinuation.[12] In the observational extension of the trial, combination of IPT and ART further decreased risk of TB by 11%.[59] However, in a pilot study of CLHIV on ART (median age 35 months) not designed to be powered for efficacy, IPT did not exert a significant protective effect on active TB (1.5 vs. 2.9 TB cases per 100 PY, IRR 0.51 [95% CI 0.15-1.75][13]. Similarly, an RCT of INH given for 96 weeks in HIV-infected and HEU infants enrolled at 91-120 days of life in South Africa and Botswana without TB exposure, did not prevent TB disease in either group.[11] Furthermore, among HEU, INH did not prevent Mtb infection as measured by a single TST at week 96. In summary, IPT is effective in adults and variably effective for preventing TB disease in HIV-infected and HEU infants, and no trial to date has been designed specifically to evaluate efficacy of IPT to prevent Mtb infection in either adults or children, including the use of both IGRA and TST as an endpoint to both maximize sensitivity of identify Mtb infection.

Study limitations

Enrollment sites are limited to two counties in western Kenya and may not be generalizable to other settings. This area was chosen due to high HIV/TB burden, as well as longstanding collaborations with study investigators in enrolling women and children from MCH/PMTCT clinics.

With non-blinded trials, there are concerns about differential reporting and clinical management, however, one of the composite endpoints (IGRA status) is assessed in the KEMRI CDC laboratory, which is blinded to INH status of the participant. This endpoint is robust and not influenced by unblinded trial design.

We have estimated a substantive INH effect (65% decrease), consistent with TB prevention literature for reduction of TB disease among TST-positive adult PLHIV,[57] but undefined for Mtb infection risk. A larger sample size may be useful if prevalence of Mtb infection is lower than anticipated or if INH is less effective in prevention of Mtb infection. We have extended post-trial follow-up to 24 months of age in an observational study to assess longer term Mtb infection incidence. This extended follow-up will allow us to better understand the timing of Mtb infection acquisition; however, results will not be included in the trial results because the extended observational period will not include receipt of IPT.

There remains a lack of a gold standard to diagnose Mtb infection;[19] both TST and IGRA are indirect measures of Mtb infection requiring both infection with Mtb as well as a functioning immune system to mount a positive test response. We have incorporated both tests within our composite primary outcome. TST at 12 months may be positive due to BCG exposure at birth rather than TB infection. Age at immunization as well as TST testing timing after BCG administration appears to affect TST reactivity, with younger age at BCG immunization associated with shorter duration of TST reactivity than in adults. In a meta-analysis of 24 studies with >240,000 participants, among participants who were BCG-vaccinated as infants, <1% were TST positive after 10 years post BCG administration, compared to 21% of participants vaccinated after their first birthday who remained TST positive after 10 years post BCG[60]. Similarly, in a recent long-term follow-up study of a BCG vs. placebo trial among Native Americans/Alaskan Natives, BCG administered after 1 year of age was associated with increased incidence of TST reactivity extending up to 55 years after vaccination.[61] Importantly, there is scant data on TST reactivity among BCG-immunized infants TST-tested during the first year of life. In a Navajo study in the US, among 250 infants immunized with BCG as newborns, 31% had TST $\geq$10mm at 3 months which reduced to zero at 9 months of age, suggesting rapid waning of BCG-associated TST responses in children receiving BCG at birth.[62] Therefore, it appears that TST testing at approximately 1 year of age among children immunized with BCG at birth is more likely to represent Mtb infection, as opposed to BCG-induced reactivity.

This study does not include qualitative work to investigate issues of adherence, though does include closed-ended questions regarding caregiver barriers and facilitators to providing prophylactic medications to HEU children.

Kenya endorsed routine IPT for PLHIV in 2014 national guidelines,[35] and counties in which this study is located have had a particularly rapid expansion of IPT as part of routine HIV care. We have described high IPT use in pregnant and early postpartum women.[63] Widespread implementation of IPT in adult PLHIV (including peripartum women) could significantly decrease TB risk in infants, making an HEU-focused TB prevention strategy less needed.

CONCLUSION

This RCT will evaluate efficacy of INH to prevent primary Mtb infection among HEU children in an endemic setting. This population is at high risk for TB exposure and progression from primary infection to TB disease and is not currently included in current TB prevention guidelines due to a lack of data.

AUTHOR CONTRIBUTIONS: GJ-S, BAR, JK, SML designed the randomized clinical trial. SML, GJ-S, BAR, TRH, LMC, JK, DM, AW, EM-O developed the study protocol. GJ-S is the principal investigator and protocol chair and TRH is the immunology principal investigator. JK is the protocol co-chair and country principal investigator. EM-O is the Pediatric Clinical TB lead. GJ-S, BAR, SML are responsible for the statistical design of the trial and data analysis of the primary outcomes. SML is the project director and drafted the statistical analysis plan overseen by BAR, the study biostatistician. SML, DM, AW, JK participated in trial implementation. TRH designed the immunologic studies and immunologic work related to the trial. SML wrote the first draft of the manuscript. All authors critically revised, read, and approved the final manuscript.

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TABLES & FIGURE LEGENDS

Figure 1 Study Schema: Aims of RCT to evaluate INH to prevent Mtb infection in HEU infants

Figure 2 Overall Study Strategy

Figure 3 CONSORT diagram

Supplemental Table 1 Overview of study visits and planned procedures

Supplemental Table 2 Primary outcome sample size estimates and power

REFERENCES

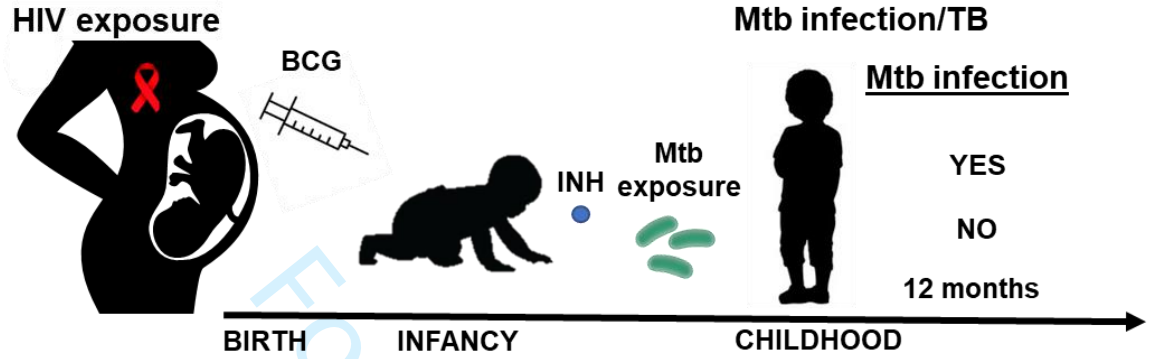
1. Cotton MF, Schaaf HS, Lottering G*, et al.* Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. *Int J Tuberc Lung Dis* 2008,**12**:225-227.
2. Madhi SA, Nachman S, Violari A*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
3. Marais BJ, Gie RP, Schaaf HS*, et al.* The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004,**8**:392-402.
4. Marais BJ, Gie RP, Schaaf HS*, et al.* The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004,**8**:278-285.
5. Gedde-Dahl T. Tuberculous infection in the light of tuberculin matriculation. *Am J Hyg* 1952,**56**:139-214.
6. Moyo S, Verver S, Mahomed H*, et al.* Age-related tuberculosis incidence and severity in children under 5 years of age in Cape Town, South Africa. *Int J Tuberc Lung Dis* 2010,**14**:149-154.
7. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 1974,**99**:131-138.
8. Starke JR. Childhood tuberculosis during the 1990s. *Pediatr Rev* 1992,**13**:343-353.
9. Donald PR, Maher D, Qazi S. A research agenda to promote the management of childhood tuberculosis within national tuberculosis programmes. *Int J Tuberc Lung Dis* 2007,**11**:370-380.
10. World Health Organization. Latent TB Infection: Updated and consolidated guidelines for programmatic management. 2018. Available at: http://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/. .
11. Madhi SA, Nachman S, Violari A*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
12. Zar HJ, Cotton MF, Strauss S*, et al.* Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007,**334**:136.
13. Gray DM, Workman LJ, Lombard CJ*, et al.* Isoniazid preventive therapy in HIV-infected children on antiretroviral therapy: a pilot study. *Int J Tuberc Lung Dis* 2014,**18**:322-327.
14. Zunza M, Gray DM, Young T*, et al.* Isoniazid for preventing tuberculosis in HIV-infected children. *Cochrane Database Syst Rev* 2017,**8**:Cd006418.
15. Cranmer LM, Kanyugo M, Jonnalagadda SR*, et al.* High prevalence of tuberculosis infection in HIV-1 exposed Kenyan infants. *Pediatr Infect Dis J* 2014,**33**:401-406.
16. Marquez C, Chamie G, Achan J*, et al.* Tuberculosis Infection in Early Childhood and the Association with HIV-exposure in HIV-uninfected Children in Rural Uganda. *Pediatr Infect Dis J* 2016,**35**:524-529.
17. Bekker A, Du Preez K, Schaaf HS*, et al.* High tuberculosis exposure among neonates in a high tuberculosis and human immunodeficiency virus burden setting. *Int J Tuberc Lung Dis* 2012,**16**:1040-1046.
18. Cotton MF, Schaaf HS, Lottering G*, et al.* Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. *Int J Tuberc Lung Dis* 2008,**12**:225-227.
19. Pai M, Denkinger CM, Kik SV*, et al.* Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clin Microbiol Rev* 2014,**27**:3-20.
20. American Academy of Pediatrics. Tuberculosis. In: *Red Book 2018-2021 Report of the Committee on Infectious Diseases; American Academy of Pediatrics*. Edited by Kimberlin DW, Brady MT, Jackson MA, Long SA. 31st Edition ed. Elk Grove Village, IL: American Academy of Pediatrics; 2018. pp. 879-853.

21. Lewinsohn DM, Leonard MK, LoBue PA*, et al.* Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. *Clin Infect Dis* 2017,**64**:111-115.
22. Pai M, Behr MA, Dowdy D*, et al.* Tuberculosis. *Nat Rev Dis Primers* 2016,**2**:16076.
23. Andrews JR, Nemes E, Tameris M*, et al.* Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study. *The Lancet Respiratory Medicine* 2017,**5**:282-290.
24. Velasco-Arnaiz E, Soriano-Arandes A, Latorre I*, et al.* Performance of Tuberculin Skin Tests and Interferon-gamma Release Assays in Children Younger Than 5 Years. *Pediatr Infect Dis J* 2018,**37**:1235-1241.
25. Mandalakas AM, van Wyk S, Kirchner HL*, et al.* Detecting tuberculosis infection in HIV-infected children: a study of diagnostic accuracy, confounding and interaction. *Pediatr Infect Dis J* 2013,**32**:e111-118.
26. Verhagen LM, Maes M, Villalba JA*, et al.* Agreement between QuantiFERON(R)-TB Gold In-Tube and the tuberculin skin test and predictors of positive test results in Warao Amerindian pediatric tuberculosis contacts. *BMC Infect Dis* 2014,**14**:383.
27. Debulpaep S, Corbiere V, Levy J*, et al.* Contribution of QuantiFERON-TB Gold-in-Tube to the Diagnosis of Mycobacterium tuberculosis Infection in Young Children in a Low TB Prevalence Country. *Front Pediatr* 2019,**7**:291.
28. Elliot C, Marais B, Williams P*, et al.* Tuberculin skin test versus interferon-gamma release assay in refugee children: A retrospective cohort study. *J Paediatr Child Health* 2018,**54**:834-839.
29. Cranmer LM, Draper HR, Mandalakas AM*, et al.* High Incidence of Tuberculosis Infection in HIV-exposed Children Exiting an Isoniazid Preventive Therapy Trial. *Pediatr Infect Dis J* 2018,**37**:e254-e256.
30. Kenya Ministry of Health. National Guidelines on Management of Tuberculosis in Children, Third Edition. 2017. Available at: https://www.chskenya.org/wp-content/uploads/2018/03/National-Guidelines-on-Management-of-Tuberculosis-in-Children.pdf.
31. Samandari T, Agizew TB, Nyirenda S*, et al.* 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011,**377**:1588-1598.
32. Rangaka MX, Wilkinson RJ, Boulle A*, et al.* Isoniazid plus antiretroviral therapy to prevent tuberculosis: a randomised double-blind, placebo-controlled trial. *Lancet* 2014,**384**:682-690.
33. World Health Organization. Global tuberculosis report 2018. 2018. Available at: http://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf?ua=1.
34. Kenya Ministry of Health. Kenya Tuberculosis Survey 2017. Available at: https://www.nltp.co.ke/survey-reports-2/.
35. Kenyan Ministry of Health, National AIDS and STI Control Program (NASCOP). Guidelines on use of antiretroviral drugs for treating and preventing HIV infection: a rapid advice. 2014. Available at: http://healthservices.uonbi.ac.ke/sites/default/files/centraladmin/healthservices/Rapid%20Advice%20Booklet%202014%2024%20June%2012%20noon_0.pdf.
36. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. 2011. Available at: http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf.
37. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children, 2nd edition. 2014. Available at: http://www.who.int/tb/publications/childtb_guidelines/en/.

38. Cobelens F, Van Deutekom H, Draayer-Jansen I*, et al.* Tuberculin skin test reactions by time of reading among Dutch travellers. *Int J Tuberc Lung Dis* 2003,**7**:758-763.
39. Tuberculin reaction size on five consecutive days. *Bull World Health Organ* 1955,**12**:189-196.
40. Nackers F, Huerga H, Espie E*, et al.* Adherence to self-administered tuberculosis treatment in a high HIV-prevalence setting: a cross-sectional survey in Homa Bay, Kenya. *PLoS One* 2012,**7**:e32140.
41. Meissner PE, Musoke P, Okwera A*, et al.* The value of urine testing for verifying adherence to anti-tuberculosis chemotherapy in children and adults in Uganda. *Int J Tuberc Lung Dis* 2002,**6**:903-908.
42. Gerona R, Wen A, Chin AT*, et al.* Quantifying Isoniazid Levels in Small Hair Samples: A Novel Method for Assessing Adherence during the Treatment of Latent and Active Tuberculosis. *PLoS One* 2016,**11**:e0155887.
43. Mave V, Chandanwale A, Kinikar A*, et al.* Isoniazid hair concentrations in children with tuberculosis: a proof of concept study. *Int J Tuberc Lung Dis* 2016,**20**:844-847.
44. Division of AIDS National Institute of Allergy and Infectious Diseases. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. 2014. Available at: http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf.
45. Harris PA, Taylor R, Thielke R*, et al.* Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009,**42**:377-381.
46. Qiagen. QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA Package Insert. 2014. Available at: http://www.quantiferon.com/irm/content/PI/QFT/PLUS/2PK-Elisa/UK.pdf.
47. Rozot V, Patrizia A, Vigano S*, et al.* Combined use of Mycobacterium tuberculosis-specific CD4 and CD8 T-cell responses is a powerful diagnostic tool of active tuberculosis. *Clin Infect Dis* 2015,**60**:432-437.
48. Armand M, Chhor V, de Lauzanne A*, et al.* Cytokine responses to quantiferon peptides in pediatric tuberculosis: a pilot study. *J Infect* 2014,**68**:62-70.
49. Kim SY, Park MS, Kim YS*, et al.* The responses of multiple cytokines following incubation of whole blood from TB patients, latently infected individuals and controls with the TB antigens ESAT-6, CFP-10 and TB7.7. *Scand J Immunol* 2012,**76**:580-586.
50. Lighter-Fisher J, Peng CH, Tse DB. Cytokine responses to QuantiFERON(R) peptides, purified protein derivative and recombinant ESAT-6 in children with tuberculosis. *Int J Tuberc Lung Dis* 2010,**14**:1548-1555.
51. Rubbo PA, Nagot N, Le Moing V*, et al.* Multicytokine detection improves latent tuberculosis diagnosis in health care workers. *J Clin Microbiol* 2012,**50**:1711-1717.
52. Biselli R, Mariotti S, Sargentini V*, et al.* Detection of interleukin-2 in addition to interferon-gamma discriminates active tuberculosis patients, latently infected individuals, and controls. *Clin Microbiol Infect* 2010,**16**:1282-1284.
53. Warr AJ, Shah JA, LaCourse SM*, et al.* Mycobacterium tuberculosis antigen-specific T-cell responses in HIV-infected mothers and their infants. In: *Keystone Symposia on Tuberculosis: Translating Scientific Findings for Clinical and Public Health Impact*. Whistler, British Columbia, Canada; April 15-19, 2018.
54. Comstock GW, Ferebee SH, Hammes LM. A controlled trial of community-wide isoniazid prophylaxis in Alaska. *Am Rev Respir Dis* 1967,**95**:935-943.
55. Comstock GW, Baum C, Snider DE, Jr. Isoniazid prophylaxis among Alaskan Eskimos: a final report of the bethel isoniazid studies. *Am Rev Respir Dis* 1979,**119**:827-830.
56. Smieja MJ, Marchetti CA, Cook DJ*, et al.* Isoniazid for preventing tuberculosis in non-HIV infected persons. *Cochrane Database Syst Rev* 2000:CD001363.

57. Akolo C, Adetifa I, Shepperd S*, et al.* Treatment of latent tuberculosis infection in HIV infected persons. *The Cochrane database of systematic reviews* 2010:CD000171.
58. Ayele HT, Mourik MS, Debray TP*, et al.* Isoniazid Prophylactic Therapy for the Prevention of Tuberculosis in HIV Infected Adults: A Systematic Review and Meta-Analysis of Randomized Trials. *PLoS One* 2015,**10**:e0142290.
59. Frigati LJ, Kranzer K, Cotton MF*, et al.* The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis in children infected with HIV in a high tuberculosis incidence setting. *Thorax* 2011,**66**:496-501.
60. Farhat M, Greenaway C, Pai M*, et al.* False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006,**10**:1192-1204.
61. Mancuso JD, Mody RM, Olsen CH*, et al.* The Long-term Effect of Bacille Calmette-Guerin Vaccination on Tuberculin Skin Testing: A 55-Year Follow-Up Study. *Chest* 2017,**152**:282-294.
62. Lifschitz M. The value of the tuberculin skin test as a screening test for tuberculosis among BCG-vaccinated children. *Pediatrics* 1965,**36**:624-627.
63. LaCourse SM, Wagner AD, Cranmer LM*, et al.* Brief Report: High Programmatic Isoniazid Preventive Therapy (IPT) Use in Pregnancy Among HIV-Infected Women. *J Acquir Immune Defic Syndr* 2019,**82**:41-45.

Figure 1: Aims of RCT to evaluate INH to prevent Mtb infection in HEU infants



DOES INH PREVENT PRIMARY MTB INFECTION in HEU?

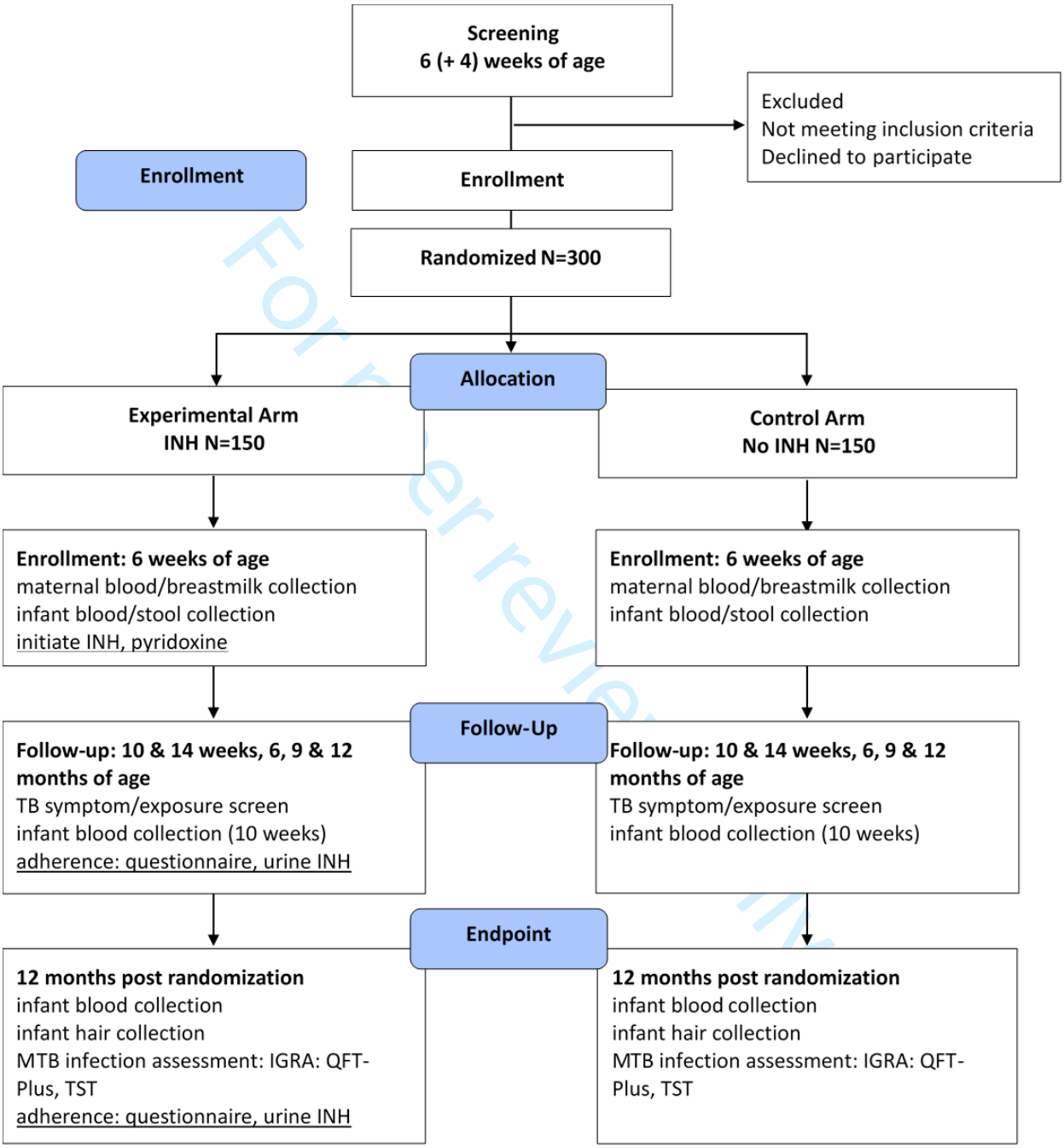
Epidemiologic (**AIM 2**) and immunologic (**AIM 3**) correlates of Mtb infection?

- Infant BCG-specific T-cell responses (blood)
- Maternal Mtb-specific T-cell responses (blood/breastmilk)
- Does INH modify these responses?

Figure 2: Overall Study Strategy

Study Design	Non-blinded randomized controlled trial
Intervention	Intervention: INH for 12 months Control group: No INH
Primary Outcomes	Aim 1: Mtb infection in HEU infants at 12 months post enrollment as measured by IGRA (QFT-Plus) and/or TST Aim 2: Epidemiologic correlates of infant Mtb infection Aim 3: Immunologic correlates of infant Mtb infection
Population	HEU infants ~6 weeks of age and their HIV-infected mothers
Exclusions	• Infants with known exposure to active TB in household • Positive HIV DNA at 6 weeks • Premature and/or birthweight 2.5 kg
Target enrollment	300 HEU infants and their HIV-infected mothers (150 each arm)
Sampling framework	Consecutive enrollment of HEU infants and their HIV-infected mothers at MCH/PMTCT clinics in western Kenya

Figure 3: CONSORT diagram



Supplemental Table 1: Overview of study visits and planned procedures				
	Enrollment 6 weeks of age	**Follow-up visit** [a]	**Endpoint 12 months post enrollment**	**TB Diagnosis**
HIV testing (per MOH)	**x**	**x**[b]	**x**	
Enrollment	**x**			
Sociodemographic survey	**x**	**x**	**x**	
Health history	**x**	**x**	**x**	
Physical exam	**x**	**x**	**x**	
TB symptom screen	**x**	**x**	**x**	
SAE assessment		**x**	**x**	
Adherence assessment via questionnaire, urine INH testing[c]		**x**	**x**	
TB exposure assessment	**x**	**x**	**x**	
Infant blood draw (PBMC/plasma, LFT[c])	**x**[d]	**x**[d]		
Maternal blood draw (PBMC/plasma)	**x**			
Maternal breastmilk collection	**x**			
Infant stool collection	**x**			
Infant TST placement			**x**[e]	**x**[e]
Infant blood draw (IGRA)			**x**[e]	**x**[e]
Infant hair collection [c]			**x**	

[a] Follow up visits will occur at 10 and 14 weeks of age, and 6, 9, and 12 months of age.

[b] Infant DNA PCR will be drawn at 6 weeks of age and HIV antibody test will be drawn at 12 months of age per Kenyan MOH guidelines.

[c] For infants randomized to INH

[d] For all infants blood will be drawn for PBMCs and plasma at the 10 week of age visit. For infants randomized to INH arm, LFTs will be drawn at baseline (6 weeks) and 10 weeks of age (1 month post INH initiation).

[e] Blood will be drawn to assess the presence of Mtb infection at study endpoint, time of TB diagnosis, and in the event of study withdrawal using QFT-plus. If blood volume is insufficient for QFT-Plus (<4ml), blood will be processed for PBMCs for flow cytometry-based assessment of Mtb infection. TST will be placed and read within 48-96 hours.

Supplemental Table 2: Primary outcome sample size estimates and power			
Power 80%, 2-sided p 0.05 1 year follow-up	Maximum HR for IPT detectable	Risk of Mtb infection after 12 months	Number per arm
0.2 risk Mtb infection after 12 months follow-up	0.5	0.2	220
	0.4	0.2	150
	0.35	0.2	120
	0.32	0.2	100
	0.2	0.2	55
0.15 risk Mtb infection after 12 months follow-up	0.5	0.15	300
	0.4	0.15	180
	0.35	0.15	150
	0.31	0.15	120
	0.2	0.15	75
0.10 risk Mtb infection after 12 months follow-up	0.5	0.1	420
	0.4	0.1	270
	0.35	0.1	220
	0.3	0.1	180
	0.2	0.1	110

Gray shaded – Expected study target

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	0
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	confirmed
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	0, 16
	5b	Name and contact information for the trial sponsor	0
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	9
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Appendix 1: Consent
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Appendix 1: Consent
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	16
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1: Consent
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Appendix 1: Consent

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

UNIVERSITY OF WASHINGTON (UW) and UNIVERSITY of NAIROBI (UoN) Collaborative Study Group

CONSENT and PARENTAL PERMISSION FORM FOR RANDOMIZED TRIAL

Preventing *Mycobacterium tuberculosis* Infection in HIV-Exposed Infants

Short Title: Infant TB Infection Prevention Study ("iTIPS")

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1. **Researcher's Statement**:

We are asking you and your child to be in a research study. The purpose of this form is to give you the information you will need to help you decide whether you and your child will be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want to be in the study or not. This process is called "informed consent." This form serves as a both as a record of your consent to be in the study and as a parental permission form. We will give you a copy of this form for your records.

The word "you" in this form refers to you and your child.

2. **What you should know about this study:**

- This form explains what would happen if you join this research study.
- Please read it carefully. Take as much time as you need.
- Please ask the research team questions about anything that is not clear.
- You can ask questions about the study at any time.
- If you choose not to be in this study, it will not affect any other care received at clinic.
- If you say 'Yes' now, you can still change your mind later.
- You can quit the study at any time.
- You would not lose benefits or be penalized if you decide not to take part in the study or to quit the study later.

3. **What is the goal of this study?**

The goal of any research study is to answer questions. We (the research team listed on the front of this form and our staff) are doing this research study to answer the following question:

- Does the medicine isoniazid (INH) decrease the risk of *Mycobacterium tuberculosis* (MTB) infection?

MTB causes the disease called tuberculosis (TB). Once infected with MTB, some people go on to get TB disease. Young children, as well as people with HIV are more likely to get TB disease because their body defenses are sometimes weak. Children, even if they are not infected with HIV, are more likely to become infected with TB if someone else in their household is also infected with both HIV and TB. INH has been

successfully used to decrease the chances of getting TB disease after MTB infection, but little is known whether it can prevent getting MTB infection in the first place.

4. **Why do I have the option of joining this study?**

You have the option to take part in this research study by being an HIV-infected mother and having a baby that was exposed to HIV.

5. **How many people will take part in this study?**

We think that about 300 mothers and their infants will take place in this research study at sites in western Kenya.

6. **If I agree to join this study, what would I need to do?**

STUDY PROCEDURES

This is what will happen if you agree to participate in this study. We will ask you to read, discuss, and sign or make your mark on this form. After this form is signed or marked, the study staff will ask you questions about you and your child's health, including HIV status, questions about your pregnancy, medications, and if you have been exposed to someone with TB. A study clinician will collect up to 5 mls of blood (teaspoon) from your infant on enrollment at 6 (+4) weeks of age, 10 weeks (+/- 4) weeks of age and 12 months after starting the study, and if your infant is diagnosed with TB disease during the study. We will also ask for a stool sample from your infant. You will also be asked to give 30 mls of breast milk and 5 mls of blood at the beginning of the study. These samples will be used to study the body's defenses against TB.

You will be placed into 1 of 2 groups. You cannot choose which group you will be placed in. You will be placed in a group by chance based on a number that has been assigned to you. The two groups you may be assigned to include either 1) INH or 2) no INH. Your child's chance of getting INH or no INH is the same, just like flipping a coin.

INH group - If your infant is assigned to the INH group, you will be asked to give your child INH daily for 1 year. Although INH is well tolerated in infants, it can be associated in very rare cases with tingling, burning, or numbness in the hands and feet. To prevent this, you will also be asked to give you child a vitamin called pyroxidine. At the beginning of the study, you will be asked how you would like to receive your child's medication every month. You can choose to come to the clinic to pick it up or have a field worker bring it to your home. Also, if your child is in the INH group we will draw blood to measure your infant's baseline liver function at enrollment (before staring INH) and after taking the medicine for 4-6 weeks. The liver is the main part of the body that filters this medication in the body.

No INH group – If your infant is assigned to the no INH group, you will still have the same study procedures performed as the INH group, such as exams and blood draws, except your child will not be given INH or pyroxidine.

These tests and exams help us find out if being in this study causes any effects that are important to know about. We use them to check on the safety of the people in this study. We also use them to learn if the experimental treatment is helping or not.

You will be asked to bring your infant to clinic to be evaluated on enrollment at approximately **6 weeks of age (enrollment), 10 weeks, 14 weeks, 6 months, 9 months, and 12 months of age**. These visits are aligned with the Kenyan recommended schedule of pediatric well child/immunization visits. Additionally, you will be asked to come to the last study visit at **12 months post-enrollment**. It is very important to come to the last visit as this is the visit when we will draw blood to see if your infant has been infected with tuberculosis. If your infant is in the INH group, you will be given enough INH and pyroxidine at each visit to last until the next visit.

Tuberculin skin test (TST). A study clinician will a small needle to put some testing material, called tuberculin, just under the skin of your infant at the end of study visit and if your infant is diagnosed with TB. We will ask you to return to the clinic in 2-3 days to check the result by measuring if there is a reaction on your skin. TST is a test that is used to diagnose MTB infection, but does not necessarily mean you have TB disease.

These tests and exams help us find out if being in this study causes any effects that are important to know about. We use them to check on the safety of the people in this study. We also use them to learn if the experimental treatment is helping or not.

Blood for genetic testing – Some of the blood drawn at the beginning of the study from your infant will be stored to do a test to check the genes (NAT2) that are related to how the body filters INH. The samples that will be tested will be chosen after the study is completed. You will not be told of the result for this test because it is for investigation only and will be done after the study is completed.

Urine and hair for INH testing – For children in the INH group, we will collect urine at the 10 weeks, 14 weeks, 6 months, 9 months, 12 months of age, and study endpoint visit (approximately 14 months of age). This urine will be used to test for INH in the clinic using a dipstick. We will also cut a small thatch of hair (approximately 30 strands) at the end of study visit. This hair will be used to measure INH. You will not be told of the result for this test on hair because it is for investigation only and will be done after the study is completed.

MEDICAL RECORD INFORMATION

We will ask for access to your and your baby's clinic and pharmacy records to find out more information about your pregnancy, delivery, and postpartum care. If you agree to give us access to your medical records, we will get information from the clinics where you received care before, during, and after delivery of your baby, including: any health problems, medication adherence and side effects, and your baby's health information. We will also record laboratory test results, like your CD4 and HIV viral load tests, and infant's HIV tests.

7. **How long would I be in this study?**

If you choose to take part in all the study visits, you and your infant would be in the study for 1 year. If you join this study, you can decide to stop **at any time, for any reason**. If you decide to stop you would need to talk with site investigators so you leave the study in a safe way.

The research study clinicians could also decide to take you out of this study. This might happen if we find out that it is not safe for you to continue in the study. It may also happen if you cannot come to enough of the study visits. If we ask you to leave the study we would always explain why and this would not hamper other care received at the facility in any way.

8. **What are the potential harms or risks if I join this study?**

There are potential harms or risks if you take part in this study. Some are common and some are rare. They are described below.

Potential Harms and Discomforts (from the most common, to the most rare):

- Local irritation due to blood draw
- Local irritation due to TST
- Maternal breast discomfort due to self-expression of breast milk
- Nausea, vomiting, stomach discomfort due to INH
- Peripheral neuropathy (numbness, tingling of the nerves in your hands and feet) due to INH
- Hepatitis (irritation of the liver which is the organ that filters the medicine INH)
- Some people feel uncomfortable answering questions about their health and their baby's health

Because this research study involves a medication that has been used primarily to treat TB disease or prevent TB disease in the past (not prevent MTB infection): we do know that in general INH is generally well tolerated by infants.

A Data Safety Monitoring Board (DSMB) will review the information from this research study. This board is made up of a group of experts responsible for looking at how people in the research study are doing. If you take part, we would tell you about any new information we learn that might affect your health or your willingness to stay in the study.

9. **What are the potential benefits if I join this study?**

Potential Benefits for You:

Being in this study might benefit you in the following ways:

- Participants will benefit from direct medical care in the long-term research group.

Potential Benefits for Others:

- All infants - those born to HIV infected mothers and those born to uninfected mothers - will benefit from a better understanding in how the body defenses protect against MTB infection
- We hope to use information we gain in this study to benefit others in regions with high tuberculosis rates.

10. **What other options do I have?**

Whether or not you decide to participate in this research study, you can continue to receive your mother-child health care at this clinic.

11. **Who is funding this study?**

The study team and/or the University of Washington and Kenyatta National Hospital are receiving financial support from the Thrasher Research Foundation Health in the United States.

12. **How would you keep my information confidential?**

We will keep your identity as a research subject confidential. Your HIV test results, your infants MTB infection test results, medical records, and responses to questions will be kept private, and no identifying information of any kind will be released to any other person or agency that is not working on this study, without your permission in writing. We will not publish or discuss in public anything that could identify you. Any specimens you provide, and your medical information will be identified by a code number. All of your information, including the link between your name and code number will be kept in a secure location at the clinic only. Once the study is completed, we will maintain the link for 5 years, after this time we will remove your name and all identifying information from the study files. Any publication of this study will not use your name or identify you personally. However, study team may share identifiable information about you in the case the study team becomes aware of possible harm to yourself or others.

Although we will make every effort to keep your information confidential, no system for protecting your confidentiality can be completely secure. It is still possible that someone could find out you were in this study and could find out information about you. Government or university staff may review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

Study records may be reviewed by:

- University of Washington, including the Institutional Review Board
- Kenyatta National Hospital and University of Nairobi, including the Ethics and Research
- Committee
- Kenya Medical Research Institute (KEMRI)

A description of this clinical trial will be available on http://www.clinical.trials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time. A copy of your consent form will be placed in your study record.

13. **Would it cost me money to be in this study?**

If you take part in this study there would be no cost to you.

14. **What if I were injured because I joined this study?**

If you think you or your infant has a medical problem or illness related to this research, contact Dr. John Kinuthia**:** +254-722-799-052 right away. He will treat you or refer you for treatment. If your child is injured as a result of being in the study, you will be offered free care at the study clinic. If you require medical care that the study clinic cannot provide, we will refer you to the appropriate organizations to receive care for the injury. The costs of the treatment may be billed to you or the National Hospital Insurance Fund (NHIF) just like other medical costs, or it may be covered by the UW's discretionary Human Subject's Assistance Program (HSAP) depending on a number of factors. The researcher may request HSAP coverage by following established procedures. If you wish to request HSAP coverage yourself, you may contact the

researchers listed on the first page, or the UW Human Subjects Division at hsdinfo@uw.edu or +1-206-543-0098. Ask the researchers if you would like information about the limits and conditions of the HSAP. The UW does not normally provide any other form of compensation for Injury. However, the law may allow you to seek payment for injury-related expenses if they are caused by malpractice or the fault of the researchers. You do not give up any legal rights by signing this consent form.

15. **Would I be paid of I join this study?**

Participants will be provided a stipend for travel.

16. **If I join the study, can I stop?**

Yes. Taking part in research is always a choice. If you decide not to be in the study, you can change your mind at any time. We ask that you tell Daniel Matemo who can be reached at +254-722-322-378.

If you choose to leave the study, it will not affect your care at the study site. You will not lose any benefits or be penalized if you choose to leave the study. We may ask you to come for a visit if you leave the study early. At that visit we may ask to collect 5 mls of blood (teaspoon) and place a TST like we would at the end of the study visit.

17. **Will my samples be used after this study is done?**

We would like to save samples of your blood and breast milk and your baby's blood,stool, and hair at the KEMRI/CDC, University of Nairobi, the University of Washington, the Fred Hutchinson Cancer Research Center, Emory University, or the University of California, San Francisco, for future HIV and/or TB related research and maternal and infant health. This may include testing for genes which may affect whether a person is more or less likely to get infections, or things that may affect infant and maternal health (mother's health during postpartum period with special emphasis on HIV-related illnesses, infant health with special emphasis on HIV-exposure, and TB exposure).

Information we get from you, and your samples, may be shared with other investigators studying HIV, TB, or mother and child health. We will not share your name or any identifying information with them. An Institutional Review Board or Independent Ethics Committee, which looks at study application to ensure the safety and rights of research participants, must approve future research studies in which we will use your or your baby's samples to obtain information about both of you. Permission from the University of Nairobi's Ethics Committee will be sought before any of these samples are used for future research. These tests are for research and are not useful for your or your baby's clinical care. Before your samples or your baby's samples leave the clinic, they will be assigned a code and your name or your baby's name will not be on them. We will store these samples for ten years after completion of the study. Storage of samples past this time period will only occur with approval from an Institutional Review Board and Ethics Committee.

If you do not want to have your or your baby's samples saved for future research, you can still be in this study and your or your baby's samples will be destroyed once testing for the study is completed. If you agree to store your or your baby's samples now, but change your mind before the end of the study, let the study staff know and we will make sure that your or your baby's samples do not get stored for future research. We will not sell your or your baby's samples. Tests done on your or your baby's samples may lead to a new invention or discovery. We have no plans to share any money or other benefits resulting from any potential invention or discovery with you.

CONSENT FOR STUDY PARTICIPATION

Subject's statement:

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have questions later about the research, I can ask one of the researchers listed above. If I have questions about my rights as a research subject, I can call the *Kenyatta National Hospital Ethics and Research Committee, at 2726300 Ext. 44102*. I give permission to the researchers to use my medical records including my baby's as described in this consent form. I will receive a copy of this consent form.

Printed name of subject	Signature or thumbprint of subject	Date
Witness Name (if caregiver illiterate)	Witness Signature	Date

CONSENT FOR SAMPLE STORAGE FOR FUTURE STUDIES INCLUDING GENETIC TESTING

Please mark, one option for each question below:

YES ___NO___ You can store **my** samples for **future research** into HIV, TB or maternal child health

YES ___NO___ You can store samples from **my baby** for **future research** into HIV, TB or maternal child health

YES ___NO___ You can store my samples for future research into HIV, TB or maternal child health **including genetic testing**

YES ___NO___ You can store samples from my baby for future research into HIV, TB or maternal child health **including genetic testing**

Printed name of subject	Signature or thumbprint of subject	Date
Witness Name (if caregiver illiterate)	Witness Signature	Date

Who do I contact if I have problems or questions?

If you ever have any questions about this study, or if you have a research-related injury, you should contact Dr. John Kinuthia. If you have questions about your rights as a research participant, you should contact the Kenyatta National Hospital Ethics and Research Committee, at 2726300 Ext. 44102.

Printed name of study staff obtaining consent	Signature	Date

Copies to: Researcher, Participant

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Infant TB Infection Prevention Study (iTIPS): a randomized trial protocol evaluating isoniazid to prevent M. tuberculosis infection in HIV-exposed uninfected children

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Infant TB Infection Prevention Study (iTIPS): a randomized trial protocol evaluating isoniazid to prevent *M. tuberculosis* infection in HIV-exposed uninfected children

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ABSTRACT

Introduction

HIV-exposed uninfected infants (HEU) in tuberculosis (TB) endemic settings are at high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in the absence of known Mtb exposure. Because infancy is a time of rapid progression from primary infection to active TB disease, it is important to define when and how TB preventive interventions exert their effect in order to develop effective prevention strategies in this high-risk population.

Methods and Analysis

We designed a non-blinded randomized controlled trial to determine efficacy of isoniazid (INH) to prevent primary Mtb infection among HEU children. Target sample size is 300 (150 infants in each arm). Children are enrolled at 6 weeks of age from maternal and child health clinics in Kenya and are randomized to receive 12 months of daily INH ~10 mg/kg plus pyridoxine or No INH. The primary endpoint is Mtb infection, assessed by interferon-gamma release assay (IGRA) QuantiFERON-TB Gold Plus (QFT-Plus) or tuberculin skin test (TST) after 12 months post-enrollment. Secondary outcomes include severe adverse events, expanded Mtb infection definition using additional QFT-Plus markers, and determining correlates of Mtb infection. Exploratory analyses include a combined outcome of TB infection, disease, and mortality, and sensitivity analyses excluding infants with baseline TB-specific responses on flow cytometry.

Ethics and Dissemination

The protocol is approved by the ethical review boards of University of Nairobi/Kenyatta National Hospital, Jaramogi Oginga Odinga Teaching and Referral Hospital, and University of Washington, and Kenya Pharmacy and Poisons Board. An external and independent Data and Safety Monitoring Board monitors adverse events. Results will be disseminated through peer-reviewed journals, presentations at local and international conferences to national and global policy makers, the local community, and participants.

Registration details: ClinicalTrials.gov NCT02613169 (Registered November 24, 2015)

Version: Protocol version 1.7, August 14, 2018.

Keywords: pediatric, tuberculosis, prevention, isoniazid, protocol

ARTICLE SUMMARY

Strengths and limitations of this study

- Most children born to mothers with HIV are HIV-exposed and uninfected (HEU), but remain at high risk of tuberculosis (TB), making them an important population in which to study TB prevention.
- Current TB prevention guidelines do not recommend routine isoniazid preventive therapy (IPT) for children (including HEU) without a known TB contact, though recent data suggest the majority of transmission to children occurs outside the household due community or unperceived household TB.
- Because of the high risk of progression to TB disease in infants, our strategy focused on prevention of primary Mtb infection, as detected by a combined endpoint of interferon-gamma release assays (IGRA) and tuberculin skin test (TST) is novel, despite known limitations of both assays.
- Widespread implementation of IPT in adult people living with HIV (including peripartum women) could significantly decrease TB risk in infants, making an HEU-focused TB prevention strategy less needed.
- Given equipoise regarding whether INH prevents Mtb infection in general, and a lack of data specifically among HEU children, a randomized controlled trial design would provide important information regarding INH efficacy for primary prevention in this high-risk population.

INTRODUCTION

HIV-exposed uninfected infants (HEU) in tuberculosis (TB) endemic settings have a high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in absence of known Mtb exposure.[1-3] Because infancy is a time of rapid progression from primary infection to active TB[4], it is important to know how TB preventive interventions exert their effects to build new strategies that adapt or extend approaches used in adults. Protecting HEU infants during this vulnerable period of immunodeficiency may provide long term benefits.

Children have a higher rate of progression from infection to active disease than adults.[5, 6] In young children who lack pre-existing adaptive immune responses, Mtb infection progresses rapidly to TB disease, and both innate and early adaptive immune responses may influence susceptibility.[5, 7-9] Virtually all childhood TB disease reflects primary disease, in contrast to adults where a significant portion of disease is due to reactivation of latent TB infection.[10] The World Health Organization (WHO) recommends isoniazid (INH) preventive therapy (IPT) be provided to people living with HIV (PLHIV) >12 months of age to prevent TB.[11] Among children living with HIV (CLHIV), three randomized controlled trials (RCTs) yielded conflicting data regarding whether INH prevents TB disease and/or mortality.[12-15] Only one evaluated HEU infants, and found no protective effect in decreasing TB disease.[12] While previous RCTs have focused on prevention of TB disease, there are scant data regarding INH impact on primary Mtb infection. Among 6-month old HEU infants in Kenya, we found 10% had evidence of Mtb infection by interferon-gamma release assays (IGRAs), suggesting a potential 20% annual cumulative incidence of Mtb infection.[16] In a South African birth cohort including 22% HIV-infected mothers, TST test conversion incidence was 11.8 per 100 child-years, with the majority of conversions occurring before 1 year of age.[17] Among Ugandan children (median age 36 months), prevalence of Mtb infection (either tuberculin skin test (TST) or positive IGRA) was 2-fold higher among HEU compared to HIV-unexposed children (HUU), with higher prevalence of TST-positivity vs. IGRA in both groups (HEU TST 27.2% vs. IGRA 6.4%, HUU TST

20.6% vs. 1.5%).[18] This suggests HEU infants have a substantial incidence of Mtb infection,[19, 20] as well as low to modest concordance between different Mtb infection measures.

There is currently no gold standard for Mtb infection diagnosis.[21] While TST is recommended in children under 5 years of age,[22, 23] false positivity due to Bacille Calmette-Guérin (BCG) vaccination can occur.[24] Data regarding IGRA performance in young children is scarce, however a recent study of BCG-immunized infants in South Africa indicated high Quantiferon interferon gamma (IFN-γ) conversion values were strongly associated with subsequent development of TB.[25] Because IGRAs offer increased specificity in the presence of recent BCG vaccine, it is plausible they could enhance the ability to measure the preventive effect of INH.[24] Cross-reactivity to non-tuberculosis mycobacteria may cause false positives in both IGRA and TST.[21] IGRA and TST agreement in children varies widely and appears affected by nutrition and HIV status, TB burden, and BCG immunization.[26-30] Recent ATS/IDSA/CDC guidelines recommend dual testing IGRA and TST for groups who are both likely to be infected and at high risk of progression to TB disease as a strategy to increase diagnostic sensitivity.[23] Reduction of specificity with this strategy may be acceptable when consequences of missed Mtb infection (and therefore missed opportunity for treatment) outweigh risks of therapy-associated adverse events. Few longitudinal studies among young infants including HEUs with serial IGRA and TST testing exist.[25, 31] A prospective infant HEU cohort using both IGRA and TST can provide an efficient approach to probe determinants of Mtb infection, more rapidly accruing endpoints (Mtb infection) than studies of TB disease. This study design can contribute unique insights regarding prevention strategies.

Kenyan guidelines mirror WHO guidelines and recommend IPT for all known TB-exposed children <5 years of age and all HIV-infected children >1 year of age regardless of TB-exposure.[11, 32] However for children <5yr without known TB exposure (including HEU), and for HIV-infected children <1 year, IPT is not recommended.[11, 32] These guidelines illustrate uncertainty regarding IPT in young children, following an RCT from South Africa/Botswana that failed to demonstrate IPT effectiveness in preventing TB disease

among CLHIV and HEU <1 year of age without known TB exposure.[12-15] However, it remains possible that among HEU children exposed to unperceived community or household TB, INH may prevent primary Mtb infection. Although data are conflicting, some adult studies have demonstrated IPT benefit in TST- or IGRA-negative adult PLHIV suggesting IPT may confer protection from Mtb infection.[33, 34]

The primary goal of this study is to determine whether INH prevents primary Mtb infection in HEU infants, to determine timing and cofactors of primary Mtb acquisition in the first year of life, and to examine the role of immune protective mechanisms in this cohort (Figure 1). This paper details the study protocol of an RCT evaluating efficacy of a 12-month course of INH to prevent Mtb infection as measured by IGRA and/or TST in HEU children enrolled at 6 weeks of age in western Kenya.

METHODS AND ANALYSIS

Study Design

The infant TB Infection Prevention Study ("iTIPS") is a 2-arm, non-blinded RCT comparing efficacy of a 12-month course of daily INH vs. No INH to prevent Mtb infection among HIV-exposed uninfected Kenyan children enrolled at 6 weeks of age (Figure 2). Eligible infants are randomized using a 1:1 allocation to INH vs. No INH (Figure 3).

Study sites

Kenya is one of 22 high TB burden countries with a generalized TB epidemic[35], with an estimated TB prevalence of 426 per 100,000.[36] This study is conducted in collaborative research sites in western Kenya embedded in Ministry of Health (MOH) maternal child health (MCH) clinics. HIV-infected mothers are followed as part of the national prevention of maternal to child transmission (PMTCT) program and currently receive Option B+ triple antiretroviral therapy.[37] Per Kenyan guidelines, all PLHIV should be screened at routine HIV care visits using symptom-based TB screening and those with negative screens are evaluated for IPT.[11, 35, 37]

Recruitment processes & eligibility criteria

We recruit HIV-infected mothers and their HEU infants from MCH/PMTCT sites. Infants aged 6 weeks (+ 4 weeks) of age are eligible for inclusion if they are born to HIV-infected mothers, with birthweight ≥2.5 kilograms, and not born premature (<37 weeks gestation). Infants with known household TB exposure, including mothers with TB diagnosed in the past year are ineligible. Infants enrolled in other TB prevention or TB vaccine studies are ineligible because these interventions might affect ascertainment of endpoints.

Randomization

Site-stratified randomization is used to allocate infants 1:1 to INH or No INH arms. Randomization numbers were generated prior to study start using STATA 14 "*ralloc*" command with resulting randomization assignment by participant ID printed on cards and placed in opaque envelopes.

Blinding

The study is non-blinded to enable prompt clinical management for any potential drug-related adverse event. IGRAs are performed in the Kenya Medical Research Institute (KEMRI) Centers for Disease Control (CDC) laboratory, which is blinded to arm. The study team administers TST and not blinded to TST result. Data monitoring by the study team is not disaggregated by study arm. The study biostatistician reviews data by arm during preparation of reports to the external Data and Safety Monitoring Board (DSMB). This data is reviewed during closed DSMB sessions, which excludes team members involved in study implementation.

Enrollment and Study procedures

Enrollment

After informed consent is obtained by study staff, household locator information, medical identification number, and cellphone contacts are obtained to facilitate tracing. On enrollment, standardized questionnaires regarding sociodemographic, clinical, obstetric and HIV-related factors, TB exposure and history, and TB symptoms (for infant, mother, and household members (by maternal report) using WHO

symptom screen[38]) are administered (Supplemental Table 1). Mothers with suspected TB are referred to the TB program for further screening. If mothers are found to have TB on enrollment, their infants are ineligible for participation and are referred to receive INH per Kenya national guidelines.

Infants undergo physical examination measuring weight, height/length, mid-upper arm circumference, and presence of BCG scar. Medical records are used to abstract data on infant birthweight, PMTCT prophylaxis, other medications, immunizations, and maternal ART regimen, viral load and CD4 cell counts.

Intervention

Isoniazid ~10 mg/kg (7-15 mg/kg) is administered once daily to infants in the INH arm for 12 months. Standardized weight-based dosing (by weight band using 100 mg scored tablets) is used, corresponding to Kenya and WHO recommendations.[32, 39] Pyridoxine is provided to children randomized to INH to decrease peripheral neuropathy risk.[32, 39] Caregivers are advised on how to pulverize isoniazid and pyridoxine to be mixed with small quantities of breastmilk, clean water, or liquid co-trimoxazole to ensure full doses are given and for ease of administration to infants. Participants in the intervention arm are administered daily INH and pyridoxine by caregivers. Infants in the control arm do not receive INH or pyridoxine.

Participant follow-up

Follow-up visits occur at 10 weeks for infants enrolled at 6 weeks of age and at 14 weeks, and 6, 9, and 12 months of age for all participants coinciding with routine Kenya pediatric visit schedule. Follow-up visits include assessment of any TB diagnosis in the mother, infant, or household member since the past visit, as well as any TB symptom in the mother, infant, and household members (by maternal report). Infants in the No INH arm found to have a known TB contact during the study are referred for IPT per Kenyan guidelines. Infants and mothers found to have TB symptoms are referred to the MOH TB Programme for further evaluation. Questionnaires regarding caregiver barriers and facilitators to providing prophylactic medications (co-trimoxazole, antiretrovirals for PMTCT, and INH [if in INH arm]) are administered at 6

month of age visit. Endpoint ascertainment occurs at a study visit 12 months post-randomization at approximately 14 months of age.

Sample collection

Infant blood for peripheral blood mononuclear cells (PBMC) and plasma are collected at baseline and visit 2 (10-14 weeks of age). At 12 months post-randomization, blood is collected for IGRA (QFT-Plus) and TST placed and read within 48-92 hours.[40, 41] Infant rectal swabs are collected at enrollment for future gut microbiome studies. Maternal breastmilk and blood for PBMC and plasma separation are collected on enrollment.

Study procedures specific to infants randomized to INH

Liver function tests (LFTs) are performed at enrollment and one month following INH initiation. Adherence is assessed by caregiver report at follow-up visits. Urine is collected at follow-up and study endpoint visits and tested using strips developed to detect INH-metabolites.[42, 43] Hair is collected at study endpoint for future assessment of isoniazid levels as a more objective adherence measure over time.[44, 45]

Safety considerations

IPT has been shown to be safe in prior RCTs and is administered routinely to TB-exposed infants.[12-15] Although routine LFT monitoring is not recommended during INH in children,[22] for this trial baseline LFTs are drawn at enrollment and one month after INH initiation for infants randomized to INH. NIH Division of AIDS (DAIDS) Table for Grading the Severity of Pediatric Adverse Events is used to grade toxicities.[46] Infants with LFTs $\leq$ grade 2 are allowed to initiate INH. Infants with baseline LFTs $\geq$ grade 3 at baseline have LFTs monitored every 2 weeks and not initiated INH until LFTs are $\leqslant$ grade 2. Children are evaluated for peripheral neuropathy using a truncated Denver Developmental test.[47] After INH initiation, if toxicity is suspected, study administered drugs are immediately discontinued and in case of concern for hepatoxicity, LFTs are repeated.

An external and independent DSMB, including experts in pediatric TB, biostatistics, and trial design, monitors severe adverse events (SAE). Summaries of SAEs are given to DSMB members during scheduled meetings. Each SAE is assigned plausibility of relatedness to study drug by study investigators. "Open" reports detailing cumulative overall SAEs are descriptive (no statistical analyses). "Closed" reports of SAE data by study arm are reviewed and the DSMB makes recommendations regarding any imbalances in safety outcomes. O'Brien-Fleming boundaries for benefit and harm are used for interim monitoring and these boundaries are provided by study statistician in closed reports. The DSMB assesses operational aspects, safety, and effectiveness and makes recommendations regarding study continuation or modifications. Futility is not considered a basis for stopping rules because of the trials' value in understanding correlates of Mtb infection in HEU infants.

Discontinuation, withdrawal, or allocation modification

Participants may withdraw at any point. Study investigators may withdraw a participant on a case-by-case basis if the intervention or study involvement poses a risk to the participant. No modification of allocation will be made. Infants who receive at least one dose of study drug will be included in per protocol analyses even in the setting of intervention discontinuation or withdrawal from study. Caregivers of infants who discontinue INH are encouraged to continue study follow-up and endpoint ascertainment.

Data collection and management

Study staff use tablets to collect de-identified data using secure password-protected Research Electronic Data Capture mobile software (REDCap, Vanderbilt University, Nashville, Tennessee, USA).[48] Data are uploaded daily from tablets to the web-based REDCap database. Study investigators will have access to the finalized dataset.

Patient and Public Involvement

Overall study results will be shared with the clinical facilities and presented to local stakeholders including MOH county and national representatives.

Outcome measures

The primary outcome is number of participants with Mtb infection by QFT-Plus assay or TST 12 months after enrollment. Similar to QuantiFERON-TB Gold (QFT) IGRA, QFT-Plus measures IFN-γ released by primarily CD4+ T helper lymphocytes after TB-specific antigen (ESAT-6 and CFP-10) stimulation. In addition, QFT-Plus measures IFN-γ released by CD8+ cytotoxic T lymphocytes, after stimulation with the same antigens, which may have increased sensitivity in children, and in populations with lower CD4 counts including HIV.[49, 50] A response of ≥0.35 IU/ml to TB antigens above the Nil response in either the primarily CD4+ response [TB1], or CD8+ response [TB2] (with Nil <8 IU/ml and a positive mitogen control) are considered positive per manufacture recommendations.[49] A TST of ≥10 mm is considered positive.[22]

Secondary outcomes include severe adverse events (Grade ≥3 per DAIDS Grading Severity of Pediatric Adverse Experiences),[46] use of IFN-γ-independent immune markers in QFT-Plus supernatants to indicate Mtb infection,[51-55] and epidemiologic and immunologic correlates of Mtb infection. Exploratory outcomes include combined endpoint of Mtb infection, TB diagnosis, and/or death, as well as sensitivity analyses of the primary outcome of Mtb infection after excluding infants with evidence of immune responses to ESAT-6 or CFP-10 at enrollment in flow cytometric analyses.

Sample size and power analysis

The primary endpoint is Mtb infection (by TST or QFT-Plus). Assuming alpha of 0.05, power of 0.80, utilizing a 2-sided test, and a 1:1 allocation ratio, with 125 infants in each arm we have power to detect at least 65% decrease in Mtb infection in INH arm vs. control if cumulative incidence of Mtb infection in control arm at 12 months is 0.2, or to detect 70-80% or higher (HR 0.3-0.2) decrease if cumulative incidence of Mtb infection in control arm is 0.15 or 0.1 (Supplemental Table 2). To account for loss to follow-up, non-adherence, and isoniazid resistance, we increased sample size by 20%, with goal

enrollment of 300 infants (150 per arm). Baseline characteristics will be compared between randomization arms to assess randomization adequacy.

Statistical methods and analysis

Primary outcome:

Modified Intention-to-treat: We will use a modified intention-to-treat approach, including all participants who underwent randomization irrespective of receiving trial medication with at least one measure of Mtb infection (QFT-Plus or TST), excluding children found to be HIV DNA positive during the study. We will compare the proportion of infants with Mtb infection (either QFT or TST positive) at 12 months between INH and No INH arms using a Chi-squared test and estimate relative risk with 95% confidence intervals using a generalized linear model (GLM) with log link and Poisson family. We will also compare cumulative incidence of Mtb infection by arm using a Cox proportional hazard regressions model.

Per protocol: We will also evaluate our primary outcome by a per protocol analysis, considering only HEU infants who took at least one dose as taking INH vs. infants who did not take any INH. We anticipate future sensitivity analyses using IPT adherence and continuation data as exposure of interest and Mtb infection as outcome.

Secondary outcome:

Safety and expanded Mtb infection outcomes: For secondary outcomes we will compare proportions of participants by arm using either Chi-squared or Fisher's exact tests as appropriate for ≥ grade 3 serious adverse events. In addition, we will conduct secondary analyses using an expanded Mtb infection definition including a positive TST, QFT-Plus, or IFN-γ-independent immune markers in QFT-Plus supernatants.

Epidemiologic and immune correlates of Mtb infection will be assessed using nested case-control studies incorporating all Mtb infections from both arms then conducting stratified analyses in each trial arm to evaluate potential cofactors modified by INH.

Exploratory outcome:

We will compare a composite endpoint of Mtb infection, TB diagnosis, and/or death between randomization groups using a Chi-squared test. Baseline assays may detect evidence of Mtb infection. We will conduct an additional exploratory analysis, incorporating data from baseline assays[56] (utilizing flow cytometry of cryopreserved PBMCs) to exclude infants with evidence of Mtb-specific immune responses to ESAT-6 or CFP-10 at enrollment also using a Chi-squared test.

ETHICS

Informed consent is obtained from caregivers. The trial protocol is approved by ethical review boards of University of Washington, University of Nairobi/Kenyatta National Hospital, and Jaramogi Oginga Odinga Teaching and Referral Hospital, and Kenya Pharmacy and Poisons Board, and registered at clinicaltrials.gov (NCT02613169). Any protocol changes will be approved by relevant ethical review boards.

HEU children are at increased risk for Mtb infection and TB disease. IPT is not routinely provided to HEU infants in Kenya without evidence of exposure to a known TB case. There is mixed evidence regarding IPT effectiveness to prevent TB disease in infants <1 year. Given potential benefits of IPT to prevent Mtb infection, heightened risk for Mtb infection in this population, and safety of intervention, there is equipoise for randomization.

Trial status

Trial recruitment and enrollment began August 15, 2016. Participant follow-up is anticipated to complete September 2019, with lab analyses anticipated to be completed in December 2019.

Dissemination plans

We will share trial results at study sites, and with regional and national policymakers. We plan on submitting final results as a peer-reviewed manuscript and will utilize International Committee of Medical Journal Editors authorship criteria. Study investigators will collaborate in writing final study results.

DISCUSSION

Isoniazid has proven benefit to treat latent TB infection and prevent active TB disease in HIV-infected and HIV-uninfected populations.[57-61] Data from adult studies in Botswana and South Africa indirectly suggest IPT may prevent Mtb infection; TST negative adult PLHIV who received IPT were protected from active TB, suggesting IPT may both prevent Mtb infection and progression to TB disease.[33, 34] IPT has had variable protective efficacy to prevent TB disease and mortality in CLHIV.[12-15] An RCT in South Africa in the pre-ART era randomized CLHIV ≥8 weeks of age to INH vs. placebo (independent of reported TB exposure) and found INH prevented TB disease by 70% and decreased mortality by 54%, leading to early trial discontinuation.[13] In the observational extension of the trial, combination of IPT and ART further decreased risk of TB by 11%.[62] However, in a pilot study of CLHIV on ART (median age 35 months) not designed to be powered for efficacy, IPT did not exert a significant protective effect on active TB (1.5 vs. 2.9 TB cases per 100 PY, IRR 0.51 [95% CI 0.15-1.75][14]. Similarly, an RCT of INH given for 96 weeks in HIV-infected and HEU infants enrolled at 91-120 days of life in South Africa and Botswana without TB exposure, did not prevent TB disease in either group.[12] Furthermore, among HEU, INH did not prevent Mtb infection as measured by a single TST at week 96. In summary, IPT is effective in adults and variably effective for preventing TB disease in HIV-infected and HEU infants, and no trial to date has been designed specifically to evaluate efficacy of IPT to prevent Mtb infection in either adults or children, including both IGRA and TST as an endpoint to both maximize sensitivity of identify Mtb infection.

Study limitations

Enrollment sites are limited to two counties in western Kenya and may not be generalizable to other settings. This area was chosen due to high HIV/TB burden, as well as longstanding collaborations with study investigators in enrolling women and children from MCH/PMTCT clinics.

With non-blinded trials, there are concerns about differential reporting and clinical management, however, one of the composite endpoint components (IGRA status) is assessed in the KEMRI CDC

laboratory, which is blinded to participant INH status. This endpoint is robust and not influenced by unblinded trial design.

We have estimated a substantive INH effect (65% decrease), consistent with TB prevention literature for reduction of TB disease among TST-positive adult PLHIV,[60] but undefined for Mtb infection risk. A larger sample size may be useful if prevalence of Mtb infection is lower than anticipated or if INH is less effective in prevention of Mtb infection. We have extended post-trial follow-up to 24 months of age in an observational study to assess longer term Mtb infection incidence. This extended follow-up will allow us to better understand timing of Mtb infection acquisition; however, results will not be included in the trial results because the extended observational period will not include receipt of IPT.

There remains a lack of a gold standard to diagnose Mtb infection;[21] both TST and IGRA are indirect measures of Mtb infection requiring both infection with Mtb as well as a functioning immune system to mount a positive test response. We have incorporated both tests within our composite primary outcome. TST at 12 months may be positive due to BCG exposure at birth rather than TB infection. Age at immunization as well as TST testing timing after BCG administration appears to affect TST reactivity, with younger age at BCG immunization associated with shorter duration of TST reactivity than in adults. In a meta-analysis of 24 studies with >240,000 participants, among participants who were BCG-vaccinated as infants, <1% were TST positive after 10 years post BCG administration, compared to 21% of participants vaccinated after their first birthday who remained TST positive after 10 years post BCG[63]. Similarly, in a recent long-term follow-up study of a BCG vs. placebo trial among Native Americans/Alaskan Natives, BCG administered after 1 year of age was associated with increased incidence of TST reactivity extending up to 55 years after vaccination.[64] Importantly, there is scant data on TST reactivity among BCG-immunized infants TST-tested during first year of life. In a Navajo study in the US, among 250 infants immunized with BCG as newborns, 31% had TST $\geq$10mm at 3 months which reduced to zero at 9 months of age, suggesting rapid waning of BCG-associated TST responses in children receiving BCG at birth.[65] Therefore, it appears

that TST testing at approximately 1 year of age among children immunized with BCG at birth is more likely to represent Mtb infection, as opposed to BCG-induced reactivity. This study does not include qualitative work to investigate issues of adherence, though does include closed-ended questions regarding caregiver barriers and facilitators to providing prophylactic medications to HEU children.

Kenya endorsed routine IPT for PLHIV in 2014 national guidelines,[37] and counties in which this study is located have had a particularly rapid expansion of IPT as part of routine HIV care. We have described high IPT use in peripartum women.[66] Widespread implementation of IPT in adult PLHIV could significantly decrease TB risk in infants, making an HEU-focused TB prevention strategy less needed. Maternal IPT use is not an exclusion criterion. Infant INH drug exposure through breastmilk is very low [67] and unlikely to exert a direct protective effect in the control arm.

Given equipoise regarding whether INH prevents Mtb infection in general, and a lack of data specifically among HEU children, an RCT design could provide important information regarding INH efficacy for primary prevention in this high-risk population.

AUTHOR CONTRIBUTIONS: GJ-S, BAR, JK, SML designed the randomized clinical trial. SML, GJ-S, BAR, TRH, LMC, JK, DM, AW, EM-O developed the study protocol. GJ-S is the principal investigator and protocol chair and TRH is the immunology principal investigator. JK is the protocol co-chair and country principal investigator. EM-O is the Pediatric Clinical TB lead. GJ-S, BAR, SML are responsible for the statistical design of the trial and data analysis of the primary outcomes. SML is the project director and drafted the statistical analysis plan overseen by BAR, the study biostatistician. SML, DM, AW, JK, JNE participated in trial implementation and manuscript preparation. TRH designed the immunologic studies and immunologic work related to the trial. SML wrote the first draft of the manuscript. All authors critically revised, read, and approved the final manuscript.

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TABLES & FIGURE LEGENDS

Figure 1 Study Schema: Aims of RCT to evaluate INH to prevent Mtb infection in HEU infants

Figure 2 Overall Study Strategy

Figure 3 CONSORT diagram

Supplemental Table 1 Overview of study visits and planned procedures

Supplemental Table 2 Primary outcome sample size estimates and power

REFERENCES

1. Cotton MF, Schaaf HS, Lottering G*, et al.* Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. *Int J Tuberc Lung Dis* 2008,**12**:225-227.
2. Madhi SA, Nachman S, Violari A*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
3. Martinez L, Lo NC, Cords O*, et al.* Paediatric tuberculosis transmission outside the household: challenging historical paradigms to inform future public health strategies. *The Lancet Respiratory Medicine* 2019,**7**:544-552.
4. Marais BJ, Gie RP, Schaaf HS*, et al.* The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004,**8**:392-402.
5. Marais BJ, Gie RP, Schaaf HS*, et al.* The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004,**8**:278-285.
6. Gedde-Dahl T. Tuberculous infection in the light of tuberculin matriculation. *Am J Hyg* 1952,**56**:139-214.
7. Moyo S, Verver S, Mahomed H*, et al.* Age-related tuberculosis incidence and severity in children under 5 years of age in Cape Town, South Africa. *Int J Tuberc Lung Dis* 2010,**14**:149-154.
8. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 1974,**99**:131-138.
9. Starke JR. Childhood tuberculosis during the 1990s. *Pediatr Rev* 1992,**13**:343-353.
10. Donald PR, Maher D, Qazi S. A research agenda to promote the management of childhood tuberculosis within national tuberculosis programmes. *Int J Tuberc Lung Dis* 2007,**11**:370-380.
11. World Health Organization. Latent TB Infection: Updated and consolidated guidelines for programmatic management. 2018. Available at: http://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/. .
12. Madhi SA, Nachman S, Violari A*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
13. Zar HJ, Cotton MF, Strauss S*, et al.* Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007,**334**:136.
14. Gray DM, Workman LJ, Lombard CJ*, et al.* Isoniazid preventive therapy in HIV-infected children on antiretroviral therapy: a pilot study. *Int J Tuberc Lung Dis* 2014,**18**:322-327.
15. Zunza M, Gray DM, Young T*, et al.* Isoniazid for preventing tuberculosis in HIV-infected children. *Cochrane Database Syst Rev* 2017,**8**:Cd006418.
16. Cranmer LM, Kanyugo M, Jonnalagadda SR*, et al.* High prevalence of tuberculosis infection in HIV-1 exposed Kenyan infants. *Pediatr Infect Dis J* 2014,**33**:401-406.
17. Martinez L, le Roux DM, Barnett W*, et al.* Tuberculin skin test conversion and primary progressive tuberculosis disease in the first 5 years of life: a birth cohort study from Cape Town, South Africa. *Lancet Child Adolesc Health* 2018,**2**:46-55.
18. Marquez C, Chamie G, Achan J*, et al.* Tuberculosis Infection in Early Childhood and the Association with HIV-exposure in HIV-uninfected Children in Rural Uganda. *Pediatr Infect Dis J* 2016,**35**:524-529.
19. Bekker A, Du Preez K, Schaaf HS*, et al.* High tuberculosis exposure among neonates in a high tuberculosis and human immunodeficiency virus burden setting. *Int J Tuberc Lung Dis* 2012,**16**:1040-1046.
20. Cotton MF, Schaaf HS, Lottering G*, et al.* Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. *Int J Tuberc Lung Dis* 2008,**12**:225-227.

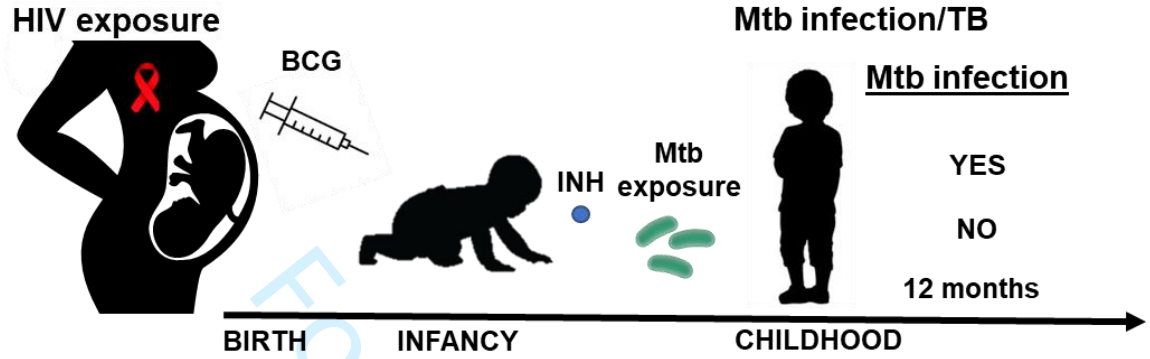
21. Pai M, Denkinger CM, Kik SV*, et al.* Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clin Microbiol Rev* 2014,**27**:3-20.
22. American Academy of Pediatrics. Tuberculosis. In: *Red Book 2018-2021 Report of the Committee on Infectious Diseases; American Academy of Pediatrics*. Edited by Kimberlin DW, Brady MT, Jackson MA, Long SA. 31st Edition ed. Elk Grove Village, IL: American Academy of Pediatrics; 2018. pp. 879-853.
23. Lewinsohn DM, Leonard MK, LoBue PA*, et al.* Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. *Clin Infect Dis* 2017,**64**:111-115.
24. Pai M, Behr MA, Dowdy D*, et al.* Tuberculosis. *Nat Rev Dis Primers* 2016,**2**:16076.
25. Andrews JR, Nemes E, Tameris M*, et al.* Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study. *The Lancet Respiratory Medicine* 2017,**5**:282-290.
26. Velasco-Arnaiz E, Soriano-Arandes A, Latorre I*, et al.* Performance of Tuberculin Skin Tests and Interferon-gamma Release Assays in Children Younger Than 5 Years. *Pediatr Infect Dis J* 2018,**37**:1235-1241.
27. Mandalakas AM, van Wyk S, Kirchner HL*, et al.* Detecting tuberculosis infection in HIV-infected children: a study of diagnostic accuracy, confounding and interaction. *Pediatr Infect Dis J* 2013,**32**:e111-118.
28. Verhagen LM, Maes M, Villalba JA*, et al.* Agreement between QuantiFERON(R)-TB Gold In-Tube and the tuberculin skin test and predictors of positive test results in Warao Amerindian pediatric tuberculosis contacts. *BMC Infect Dis* 2014,**14**:383.
29. Debulpaep S, Corbiere V, Levy J*, et al.* Contribution of QuantiFERON-TB Gold-in-Tube to the Diagnosis of Mycobacterium tuberculosis Infection in Young Children in a Low TB Prevalence Country. *Front Pediatr* 2019,**7**:291.
30. Elliot C, Marais B, Williams P*, et al.* Tuberculin skin test versus interferon-gamma release assay in refugee children: A retrospective cohort study. *J Paediatr Child Health* 2018,**54**:834-839.
31. Cranmer LM, Draper HR, Mandalakas AM*, et al.* High Incidence of Tuberculosis Infection in HIV-exposed Children Exiting an Isoniazid Preventive Therapy Trial. *Pediatr Infect Dis J* 2018,**37**:e254-e256.
32. Kenya Ministry of Health. National Guidelines on Management of Tuberculosis in Children, Third Edition. 2017. Available at: https://www.chskenya.org/wp-content/uploads/2018/03/National-Guidelines-on-Management-of-Tuberculosis-in-Children.pdf.
33. Samandari T, Agizew TB, Nyirenda S*, et al.* 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011,**377**:1588-1598.
34. Rangaka MX, Wilkinson RJ, Boulle A*, et al.* Isoniazid plus antiretroviral therapy to prevent tuberculosis: a randomised double-blind, placebo-controlled trial. *Lancet* 2014,**384**:682-690.
35. World Health Organization. Global tuberculosis report 2018. 2018. Available at: http://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf?ua=1.
36. Kenya Ministry of Health. Kenya Tuberculosis Survey 2017. Available at: https://www.nltp.co.ke/survey-reports-2/.
37. Kenyan Ministry of Health, National AIDS and STI Control Program (NASCOP). Guidelines on use of antiretroviral drugs for treating and preventing HIV infection: a rapid advice. 2014. Available at: http://healthservices.uonbi.ac.ke/sites/default/files/centraladmin/healthservices/Rapid%20Advice%20Booklet%202014%2024%20June%2012%20noon_0.pdf.

38. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. 2011. Available at: http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf.
39. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children, 2nd edition. 2014. Available at: http://www.who.int/tb/publications/childtb_guidelines/en/.
40. Cobelens F, Van Deutekom H, Draayer-Jansen I*, et al.* Tuberculin skin test reactions by time of reading among Dutch travellers. *Int J Tuberc Lung Dis* 2003,**7**:758-763.
41. Tuberculin reaction size on five consecutive days. *Bull World Health Organ* 1955,**12**:189-196.
42. Nackers F, Huerga H, Espie E*, et al.* Adherence to self-administered tuberculosis treatment in a high HIV-prevalence setting: a cross-sectional survey in Homa Bay, Kenya. *PLoS One* 2012,**7**:e32140.
43. Meissner PE, Musoke P, Okwera A*, et al.* The value of urine testing for verifying adherence to anti-tuberculosis chemotherapy in children and adults in Uganda. *Int J Tuberc Lung Dis* 2002,**6**:903-908.
44. Gerona R, Wen A, Chin AT*, et al.* Quantifying Isoniazid Levels in Small Hair Samples: A Novel Method for Assessing Adherence during the Treatment of Latent and Active Tuberculosis. *PLoS One* 2016,**11**:e0155887.
45. Mave V, Chandanwale A, Kinikar A*, et al.* Isoniazid hair concentrations in children with tuberculosis: a proof of concept study. *Int J Tuberc Lung Dis* 2016,**20**:844-847.
46. Division of AIDS National Institute of Allergy and Infectious Diseases. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. 2014. Available at: http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf.
47. IMPAACT P1041 trial protocol. 2007. Available at: https://impaactnetwork.org/studies/P1041.asp.
48. Harris PA, Taylor R, Thielke R*, et al.* Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009,**42**:377-381.
49. Qiagen. QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA Package Insert. 2014. Available at: http://www.quantiferon.com/irm/content/PI/QFT/PLUS/2PK-Elisa/UK.pdf.
50. Rozot V, Patrizia A, Vigano S*, et al.* Combined use of Mycobacterium tuberculosis-specific CD4 and CD8 T-cell responses is a powerful diagnostic tool of active tuberculosis. *Clin Infect Dis* 2015,**60**:432-437.
51. Armand M, Chhor V, de Lauzanne A*, et al.* Cytokine responses to quantiferon peptides in pediatric tuberculosis: a pilot study. *J Infect* 2014,**68**:62-70.
52. Kim SY, Park MS, Kim YS*, et al.* The responses of multiple cytokines following incubation of whole blood from TB patients, latently infected individuals and controls with the TB antigens ESAT-6, CFP-10 and TB7.7. *Scand J Immunol* 2012,**76**:580-586.
53. Lighter-Fisher J, Peng CH, Tse DB. Cytokine responses to QuantiFERON(R) peptides, purified protein derivative and recombinant ESAT-6 in children with tuberculosis. *Int J Tuberc Lung Dis* 2010,**14**:1548-1555.
54. Rubbo PA, Nagot N, Le Moing V*, et al.* Multicytokine detection improves latent tuberculosis diagnosis in health care workers. *J Clin Microbiol* 2012,**50**:1711-1717.
55. Biselli R, Mariotti S, Sargentini V*, et al.* Detection of interleukin-2 in addition to interferon-gamma discriminates active tuberculosis patients, latently infected individuals, and controls. *Clin Microbiol Infect* 2010,**16**:1282-1284.
56. Warr AJ, Shah JA, LaCourse SM*, et al.* Mycobacterium tuberculosis antigen-specific T-cell responses in HIV-infected mothers and their infants. In: *Keystone Symposia on Tuberculosis:*

Translating Scientific Findings for Clinical and Public Health Impact. Whistler, British Columbia, Canada; April 15-19, 2018.

57. Comstock GW, Ferebee SH, Hammes LM. A controlled trial of community-wide isoniazid prophylaxis in Alaska. *Am Rev Respir Dis* 1967,**95**:935-943.
58. Comstock GW, Baum C, Snider DE, Jr. Isoniazid prophylaxis among Alaskan Eskimos: a final report of the bethel isoniazid studies. *Am Rev Respir Dis* 1979,**119**:827-830.
59. Smieja MJ, Marchetti CA, Cook DJ*, et al.* Isoniazid for preventing tuberculosis in non-HIV infected persons. *Cochrane Database Syst Rev* 2000:CD001363.
60. Akolo C, Adetifa I, Shepperd S*, et al.* Treatment of latent tuberculosis infection in HIV infected persons. *The Cochrane database of systematic reviews* 2010:CD000171.
61. Ayele HT, Mourik MS, Debray TP*, et al.* Isoniazid Prophylactic Therapy for the Prevention of Tuberculosis in HIV Infected Adults: A Systematic Review and Meta-Analysis of Randomized Trials. *PLoS One* 2015,**10**:e0142290.
62. Frigati LJ, Kranzer K, Cotton MF*, et al.* The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis in children infected with HIV in a high tuberculosis incidence setting. *Thorax* 2011,**66**:496-501.
63. Farhat M, Greenaway C, Pai M*, et al.* False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006,**10**:1192-1204.
64. Mancuso JD, Mody RM, Olsen CH*, et al.* The Long-term Effect of Bacille Calmette-Guerin Vaccination on Tuberculin Skin Testing: A 55-Year Follow-Up Study. *Chest* 2017,**152**:282-294.
65. Lifschitz M. The value of the tuberculin skin test as a screening test for tuberculosis among BCG-vaccinated children. *Pediatrics* 1965,**36**:624-627.
66. LaCourse SM, Wagner AD, Cranmer LM*, et al.* Brief Report: High Programmatic Isoniazid Preventive Therapy (IPT) Use in Pregnancy Among HIV-Infected Women. *J Acquir Immune Defic Syndr* 2019,**82**:41-45.
67. Garessus EDG, Mielke H, Gundert-Remy U. Exposure of Infants to Isoniazid via Breast Milk After Maternal Drug Intake of Recommended Doses Is Clinically Insignificant Irrespective of Metaboliser Status. A Physiologically-Based Pharmacokinetic (PBPK) Modelling Approach to Estimate Drug Exposure of Infants via Breast-Feeding. *Front Pharmacol* 2019,**10**:5.

Figure 1: Aims of RCT to evaluate INH to prevent Mtb infection in HEU infants



DOES INH PREVENT PRIMARY MTB INFECTION in HEU?

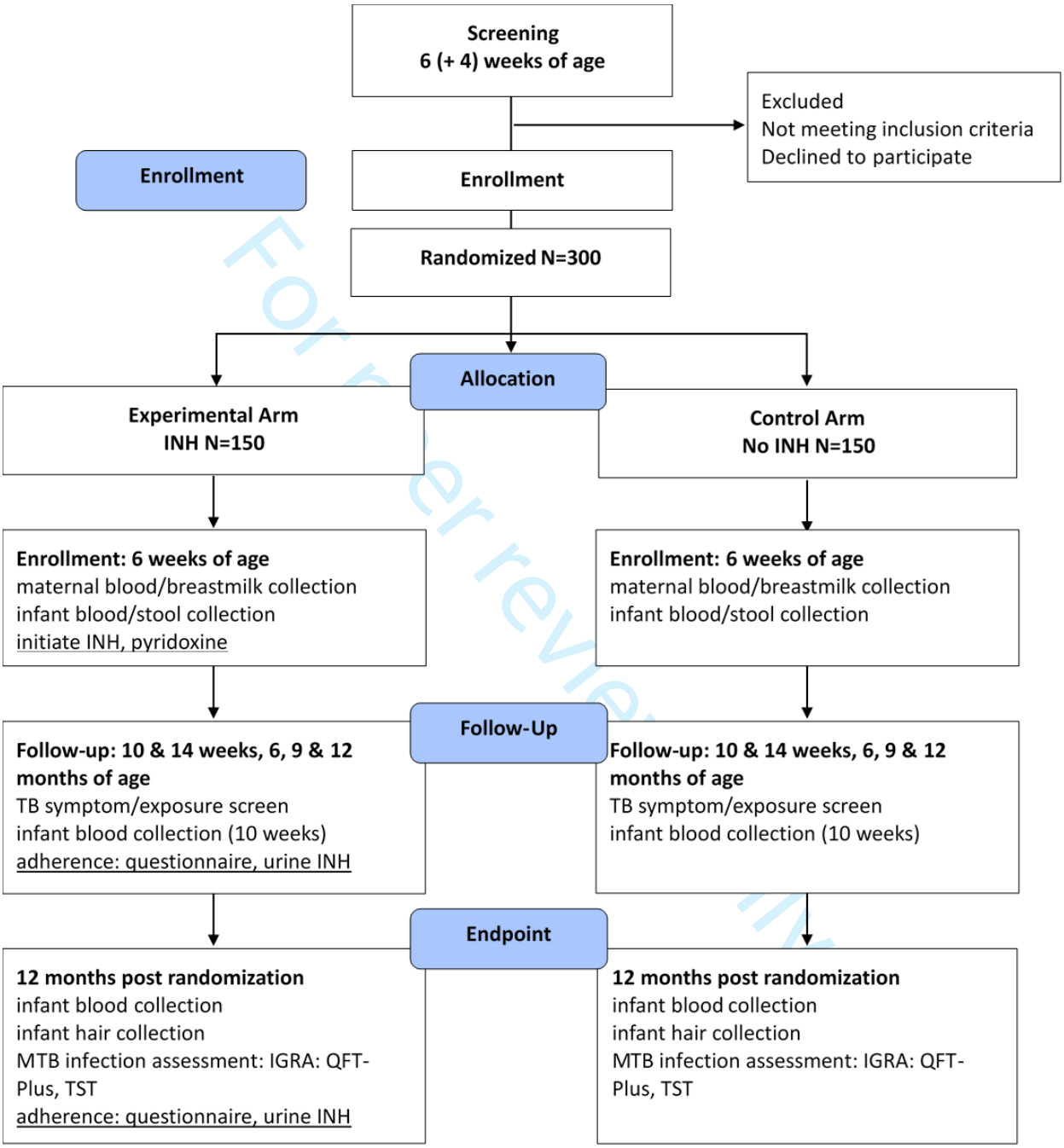
Epidemiologic (**AIM 2**) and immunologic (**AIM 3**) correlates of Mtb infection?

- Infant BCG-specific T-cell responses (blood)
- Maternal Mtb-specific T-cell responses (blood/breastmilk)
- Does INH modify these responses?

Figure 2: Overall Study Strategy

Study Design	Non-blinded randomized controlled trial
Intervention	Intervention: INH for 12 months Control group: No INH
Primary Outcomes	Aim 1: Mtb infection in HEU infants at 12 months post enrollment as measured by IGRA (QFT-Plus) and/or TST Aim 2: Epidemiologic correlates of infant Mtb infection Aim 3: Immunologic correlates of infant Mtb infection
Population	HEU infants ~6 weeks of age and their HIV-infected mothers
Exclusions	• Infants with known exposure to active TB in household • Positive HIV DNA at 6 weeks • Premature and/or birthweight 2.5 kg
Target enrollment	300 HEU infants and their HIV-infected mothers (150 each arm)
Sampling framework	Consecutive enrollment of HEU infants and their HIV-infected mothers at MCH/PMTCT clinics in western Kenya

Figure 3: CONSORT diagram



Supplemental Table 1: Overview of study visits and planned procedures				
	Enrollment 6 weeks of age	**Follow-up visit** [a]	**Endpoint 12 months post enrollment**	**TB Diagnosis**
HIV testing (per MOH)	**x**	**x**[b]	**x**	
Enrollment	**x**			
Sociodemographic survey	**x**	**x**	**x**	
Health history	**x**	**x**	**x**	
Physical exam	**x**	**x**	**x**	
TB symptom screen	**x**	**x**	**x**	
SAE assessment		**x**	**x**	
Adherence assessment via questionnaire, urine INH testing[c]		**x**	**x**	
TB exposure assessment	**x**	**x**	**x**	
Infant blood draw (PBMC/plasma, LFT[c])	**x**[d]	**x**[d]		
Maternal blood draw (PBMC/plasma)	**x**			
Maternal breastmilk collection	**x**			
Infant stool collection	**x**			
Infant TST placement			**x**[e]	**x**[e]
Infant blood draw (IGRA)			**x**[e]	**x**[e]
Infant hair collection [c]			**x**	

[a] Follow up visits will occur at 10 and 14 weeks of age, and 6, 9, and 12 months of age.

[b] Infant DNA PCR will be drawn at 6 weeks of age and HIV antibody test will be drawn at 12 months of age per Kenyan MOH guidelines.

[c] For infants randomized to INH

[d] For all infants blood will be drawn for PBMCs and plasma at the 10 week of age visit. For infants randomized to INH arm, LFTs will be drawn at baseline (6 weeks) and 10 weeks of age (1 month post INH initiation).

[e] Blood will be drawn to assess the presence of Mtb infection at study endpoint, time of TB diagnosis, and in the event of study withdrawal using QFT-plus. If blood volume is insufficient for QFT-Plus (<4ml), blood will be processed for PBMCs for flow cytometry-based assessment of Mtb infection. TST will be placed and read within 48-96 hours.

Supplemental Table 2: Primary outcome sample size estimates and power			
Power 80%, 2-sided p 0.05 1 year follow-up	Maximum HR for IPT detectable	Risk of Mtb infection after 12 months	Number per arm
0.2 risk Mtb infection after 12 months follow-up	0.5	0.2	220
	0.4	0.2	150
	0.35	0.2	120
	0.32	0.2	100
	0.2	0.2	55
0.15 risk Mtb infection after 12 months follow-up	0.5	0.15	300
	0.4	0.15	180
	0.35	0.15	150
	0.31	0.15	120
	0.2	0.15	75
0.10 risk Mtb infection after 12 months follow-up	0.5	0.1	420
	0.4	0.1	270
	0.35	0.1	220
	0.3	0.1	180
	0.2	0.1	110

Gray shaded – Expected study target

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	0
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	confirmed
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	0, 16
	5b	Name and contact information for the trial sponsor	0
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	9
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Appendix 1: Consent
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Appendix 1: Consent
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	16
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1: Consent
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Appendix 1: Consent

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

UNIVERSITY OF WASHINGTON (UW) and UNIVERSITY of NAIROBI (UoN) Collaborative Study Group

CONSENT and PARENTAL PERMISSION FORM FOR RANDOMIZED TRIAL

Preventing *Mycobacterium tuberculosis* Infection in HIV-Exposed Infants

Short Title: Infant TB Infection Prevention Study ("iTIPS")

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Emergency telephone number: Dr. John Kinuthia**:** +254-722-799-052

1. **Researcher's Statement**:

We are asking you and your child to be in a research study. The purpose of this form is to give you the information you will need to help you decide whether you and your child will be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want to be in the study or not. This process is called "informed consent." This form serves as a both as a record of your consent to be in the study and as a parental permission form. We will give you a copy of this form for your records.

The word "you" in this form refers to you and your child.

2. **What you should know about this study:**

- This form explains what would happen if you join this research study.
- Please read it carefully. Take as much time as you need.
- Please ask the research team questions about anything that is not clear.
- You can ask questions about the study at any time.
- If you choose not to be in this study, it will not affect any other care received at clinic.
- If you say 'Yes' now, you can still change your mind later.
- You can quit the study at any time.
- You would not lose benefits or be penalized if you decide not to take part in the study or to quit the study later.

3. **What is the goal of this study?**

The goal of any research study is to answer questions. We (the research team listed on the front of this form and our staff) are doing this research study to answer the following question:

- Does the medicine isoniazid (INH) decrease the risk of *Mycobacterium tuberculosis* (MTB) infection?

MTB causes the disease called tuberculosis (TB). Once infected with MTB, some people go on to get TB disease. Young children, as well as people with HIV are more likely to get TB disease because their body defenses are sometimes weak. Children, even if they are not infected with HIV, are more likely to become infected with TB if someone else in their household is also infected with both HIV and TB. INH has been

successfully used to decrease the chances of getting TB disease after MTB infection, but little is known whether it can prevent getting MTB infection in the first place.

4. **Why do I have the option of joining this study?**

You have the option to take part in this research study by being an HIV-infected mother and having a baby that was exposed to HIV.

5. **How many people will take part in this study?**

We think that about 300 mothers and their infants will take place in this research study at sites in western Kenya.

6. **If I agree to join this study, what would I need to do?**

STUDY PROCEDURES

This is what will happen if you agree to participate in this study. We will ask you to read, discuss, and sign or make your mark on this form. After this form is signed or marked, the study staff will ask you questions about you and your child's health, including HIV status, questions about your pregnancy, medications, and if you have been exposed to someone with TB. A study clinician will collect up to 5 mls of blood (teaspoon) from your infant on enrollment at 6 (+4) weeks of age, 10 weeks (+/- 4) weeks of age and 12 months after starting the study, and if your infant is diagnosed with TB disease during the study. We will also ask for a stool sample from your infant. You will also be asked to give 30 mls of breast milk and 5 mls of blood at the beginning of the study. These samples will be used to study the body's defenses against TB.

You will be placed into 1 of 2 groups. You cannot choose which group you will be placed in. You will be placed in a group by chance based on a number that has been assigned to you. The two groups you may be assigned to include either 1) INH or 2) no INH. Your child's chance of getting INH or no INH is the same, just like flipping a coin.

INH group - If your infant is assigned to the INH group, you will be asked to give your child INH daily for 1 year. Although INH is well tolerated in infants, it can be associated in very rare cases with tingling, burning, or numbness in the hands and feet. To prevent this, you will also be asked to give you child a vitamin called pyroxidine. At the beginning of the study, you will be asked how you would like to receive your child's medication every month. You can choose to come to the clinic to pick it up or have a field worker bring it to your home. Also, if your child is in the INH group we will draw blood to measure your infant's baseline liver function at enrollment (before staring INH) and after taking the medicine for 4-6 weeks. The liver is the main part of the body that filters this medication in the body.

No INH group – If your infant is assigned to the no INH group, you will still have the same study procedures performed as the INH group, such as exams and blood draws, except your child will not be given INH or pyroxidine.

These tests and exams help us find out if being in this study causes any effects that are important to know about. We use them to check on the safety of the people in this study. We also use them to learn if the experimental treatment is helping or not.

You will be asked to bring your infant to clinic to be evaluated on enrollment at approximately **6 weeks of age (enrollment), 10 weeks, 14 weeks, 6 months, 9 months, and 12 months of age**. These visits are aligned with the Kenyan recommended schedule of pediatric well child/immunization visits. Additionally, you will be asked to come to the last study visit at **12 months post-enrollment**. It is very important to come to the last visit as this is the visit when we will draw blood to see if your infant has been infected with tuberculosis. If your infant is in the INH group, you will be given enough INH and pyroxidine at each visit to last until the next visit.

Tuberculin skin test (TST). A study clinician will a small needle to put some testing material, called tuberculin, just under the skin of your infant at the end of study visit and if your infant is diagnosed with TB. We will ask you to return to the clinic in 2-3 days to check the result by measuring if there is a reaction on your skin. TST is a test that is used to diagnose MTB infection, but does not necessarily mean you have TB disease.

These tests and exams help us find out if being in this study causes any effects that are important to know about. We use them to check on the safety of the people in this study. We also use them to learn if the experimental treatment is helping or not.

Blood for genetic testing – Some of the blood drawn at the beginning of the study from your infant will be stored to do a test to check the genes (NAT2) that are related to how the body filters INH. The samples that will be tested will be chosen after the study is completed. You will not be told of the result for this test because it is for investigation only and will be done after the study is completed.

Urine and hair for INH testing – For children in the INH group, we will collect urine at the 10 weeks, 14 weeks, 6 months, 9 months, 12 months of age, and study endpoint visit (approximately 14 months of age). This urine will be used to test for INH in the clinic using a dipstick. We will also cut a small thatch of hair (approximately 30 strands) at the end of study visit. This hair will be used to measure INH. You will not be told of the result for this test on hair because it is for investigation only and will be done after the study is completed.

MEDICAL RECORD INFORMATION

We will ask for access to your and your baby's clinic and pharmacy records to find out more information about your pregnancy, delivery, and postpartum care. If you agree to give us access to your medical records, we will get information from the clinics where you received care before, during, and after delivery of your baby, including: any health problems, medication adherence and side effects, and your baby's health information. We will also record laboratory test results, like your CD4 and HIV viral load tests, and infant's HIV tests.

7. **How long would I be in this study?**

If you choose to take part in all the study visits, you and your infant would be in the study for 1 year. If you join this study, you can decide to stop **at any time, for any reason**. If you decide to stop you would need to talk with site investigators so you leave the study in a safe way.

The research study clinicians could also decide to take you out of this study. This might happen if we find out that it is not safe for you to continue in the study. It may also happen if you cannot come to enough of the study visits. If we ask you to leave the study we would always explain why and this would not hamper other care received at the facility in any way.

8. **What are the potential harms or risks if I join this study?**

There are potential harms or risks if you take part in this study. Some are common and some are rare. They are described below.

Potential Harms and Discomforts (from the most common, to the most rare):

- Local irritation due to blood draw
- Local irritation due to TST
- Maternal breast discomfort due to self-expression of breast milk
- Nausea, vomiting, stomach discomfort due to INH
- Peripheral neuropathy (numbness, tingling of the nerves in your hands and feet) due to INH
- Hepatitis (irritation of the liver which is the organ that filters the medicine INH)
- Some people feel uncomfortable answering questions about their health and their baby's health

Because this research study involves a medication that has been used primarily to treat TB disease or prevent TB disease in the past (not prevent MTB infection): we do know that in general INH is generally well tolerated by infants.

A Data Safety Monitoring Board (DSMB) will review the information from this research study. This board is made up of a group of experts responsible for looking at how people in the research study are doing. If you take part, we would tell you about any new information we learn that might affect your health or your willingness to stay in the study.

9. **What are the potential benefits if I join this study?**

Potential Benefits for You:

Being in this study might benefit you in the following ways:

- Participants will benefit from direct medical care in the long-term research group.

Potential Benefits for Others:

- All infants - those born to HIV infected mothers and those born to uninfected mothers - will benefit from a better understanding in how the body defenses protect against MTB infection
- We hope to use information we gain in this study to benefit others in regions with high tuberculosis rates.

10. **What other options do I have?**

Whether or not you decide to participate in this research study, you can continue to receive your mother-child health care at this clinic.

11. **Who is funding this study?**

The study team and/or the University of Washington and Kenyatta National Hospital are receiving financial support from the Thrasher Research Foundation Health in the United States.

12. **How would you keep my information confidential?**

We will keep your identity as a research subject confidential. Your HIV test results, your infants MTB infection test results, medical records, and responses to questions will be kept private, and no identifying information of any kind will be released to any other person or agency that is not working on this study, without your permission in writing. We will not publish or discuss in public anything that could identify you. Any specimens you provide, and your medical information will be identified by a code number. All of your information, including the link between your name and code number will be kept in a secure location at the clinic only. Once the study is completed, we will maintain the link for 5 years, after this time we will remove your name and all identifying information from the study files. Any publication of this study will not use your name or identify you personally. However, study team may share identifiable information about you in the case the study team becomes aware of possible harm to yourself or others.

Although we will make every effort to keep your information confidential, no system for protecting your confidentiality can be completely secure. It is still possible that someone could find out you were in this study and could find out information about you. Government or university staff may review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

Study records may be reviewed by:

- University of Washington, including the Institutional Review Board
- Kenyatta National Hospital and University of Nairobi, including the Ethics and Research
- Committee
- Kenya Medical Research Institute (KEMRI)

A description of this clinical trial will be available on http://www.clinical.trials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time. A copy of your consent form will be placed in your study record.

13. **Would it cost me money to be in this study?**

If you take part in this study there would be no cost to you.

14. **What if I were injured because I joined this study?**

If you think you or your infant has a medical problem or illness related to this research, contact Dr. John Kinuthia**:** +254-722-799-052 right away. He will treat you or refer you for treatment. If your child is injured as a result of being in the study, you will be offered free care at the study clinic. If you require medical care that the study clinic cannot provide, we will refer you to the appropriate organizations to receive care for the injury. The costs of the treatment may be billed to you or the National Hospital Insurance Fund (NHIF) just like other medical costs, or it may be covered by the UW's discretionary Human Subject's Assistance Program (HSAP) depending on a number of factors. The researcher may request HSAP coverage by following established procedures. If you wish to request HSAP coverage yourself, you may contact the

researchers listed on the first page, or the UW Human Subjects Division at hsdinfo@uw.edu or +1-206-543-0098. Ask the researchers if you would like information about the limits and conditions of the HSAP. The UW does not normally provide any other form of compensation for Injury. However, the law may allow you to seek payment for injury-related expenses if they are caused by malpractice or the fault of the researchers. You do not give up any legal rights by signing this consent form.

15. **Would I be paid of I join this study?**

Participants will be provided a stipend for travel.

16. **If I join the study, can I stop?**

Yes. Taking part in research is always a choice. If you decide not to be in the study, you can change your mind at any time. We ask that you tell Daniel Matemo who can be reached at +254-722-322-378.

If you choose to leave the study, it will not affect your care at the study site. You will not lose any benefits or be penalized if you choose to leave the study. We may ask you to come for a visit if you leave the study early. At that visit we may ask to collect 5 mls of blood (teaspoon) and place a TST like we would at the end of the study visit.

17. **Will my samples be used after this study is done?**

We would like to save samples of your blood and breast milk and your baby's blood,stool, and hair at the KEMRI/CDC, University of Nairobi, the University of Washington, the Fred Hutchinson Cancer Research Center, Emory University, or the University of California, San Francisco, for future HIV and/or TB related research and maternal and infant health. This may include testing for genes which may affect whether a person is more or less likely to get infections, or things that may affect infant and maternal health (mother's health during postpartum period with special emphasis on HIV-related illnesses, infant health with special emphasis on HIV-exposure, and TB exposure).

Information we get from you, and your samples, may be shared with other investigators studying HIV, TB, or mother and child health. We will not share your name or any identifying information with them. An Institutional Review Board or Independent Ethics Committee, which looks at study application to ensure the safety and rights of research participants, must approve future research studies in which we will use your or your baby's samples to obtain information about both of you. Permission from the University of Nairobi's Ethics Committee will be sought before any of these samples are used for future research. These tests are for research and are not useful for your or your baby's clinical care. Before your samples or your baby's samples leave the clinic, they will be assigned a code and your name or your baby's name will not be on them. We will store these samples for ten years after completion of the study. Storage of samples past this time period will only occur with approval from an Institutional Review Board and Ethics Committee.

If you do not want to have your or your baby's samples saved for future research, you can still be in this study and your or your baby's samples will be destroyed once testing for the study is completed. If you agree to store your or your baby's samples now, but change your mind before the end of the study, let the study staff know and we will make sure that your or your baby's samples do not get stored for future research. We will not sell your or your baby's samples. Tests done on your or your baby's samples may lead to a new invention or discovery. We have no plans to share any money or other benefits resulting from any potential invention or discovery with you.

CONSENT FOR STUDY PARTICIPATION

Subject's statement:

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have questions later about the research, I can ask one of the researchers listed above. If I have questions about my rights as a research subject, I can call the *Kenyatta National Hospital Ethics and Research Committee, at 2726300 Ext. 44102*. I give permission to the researchers to use my medical records including my baby's as described in this consent form. I will receive a copy of this consent form.

Printed name of subject	Signature or thumbprint of subject	Date
Witness Name (if caregiver illiterate)	Witness Signature	Date

CONSENT FOR SAMPLE STORAGE FOR FUTURE STUDIES INCLUDING GENETIC TESTING

Please mark, one option for each question below:

YES ___NO___ You can store **my** samples for **future research** into HIV, TB or maternal child health

YES ___NO___ You can store samples from **my baby** for **future research** into HIV, TB or maternal child health

YES ___NO___ You can store my samples for future research into HIV, TB or maternal child health **including genetic testing**

YES ___NO___ You can store samples from my baby for future research into HIV, TB or maternal child health **including genetic testing**

Printed name of subject	Signature or thumbprint of subject	Date
Witness Name (if caregiver illiterate)	Witness Signature	Date

Who do I contact if I have problems or questions?

If you ever have any questions about this study, or if you have a research-related injury, you should contact Dr. John Kinuthia. If you have questions about your rights as a research participant, you should contact the Kenyatta National Hospital Ethics and Research Committee, at 2726300 Ext. 44102.

Printed name of study staff obtaining consent	Signature	Date

Copies to: Researcher, Participant

BMJ Open

Infant TB Infection Prevention Study (iTIPS): a randomized trial protocol evaluating isoniazid to prevent M. tuberculosis infection in HIV-exposed uninfected children

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Infant TB Infection Prevention Study (iTIPS): a randomized trial protocol evaluating isoniazid to prevent *M. tuberculosis* infection in HIV-exposed uninfected children

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ABSTRACT

Introduction

HIV-exposed uninfected infants (HEU) in tuberculosis (TB) endemic settings are at high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in the absence of known Mtb exposure. Because infancy is a time of rapid progression from primary infection to active TB disease, it is important to define when and how TB preventive interventions exert their effect in order to develop effective prevention strategies in this high-risk population.

Methods and Analysis

We designed a non-blinded randomized controlled trial to determine efficacy of isoniazid (INH) to prevent primary Mtb infection among HEU children. Target sample size is 300 (150 infants in each arm). Children are enrolled at 6 weeks of age from maternal and child health clinics in Kenya and are randomized to receive 12 months of daily INH ~10 mg/kg plus pyridoxine or no INH. The primary endpoint is Mtb infection, assessed by interferon-gamma release assay (IGRA) QuantiFERON-TB Gold Plus (QFT-Plus) or tuberculin skin test (TST) after 12 months post-enrollment. Secondary outcomes include severe adverse events, expanded Mtb infection definition using additional QFT-Plus markers, and determining correlates of Mtb infection. Exploratory analyses include a combined outcome of TB infection, disease, and mortality, and sensitivity analyses excluding infants with baseline TB-specific responses on flow cytometry.

Ethics and Dissemination

The protocol is approved by the ethical review boards of University of Nairobi/Kenyatta National Hospital, Jaramogi Oginga Odinga Teaching and Referral Hospital, and University of Washington, and Kenya Pharmacy and Poisons Board. An external and independent Data and Safety Monitoring Board monitors adverse events. Results will be disseminated through peer-reviewed journals, presentations at local and international conferences to national and global policy makers, the local community, and participants.

Registration details: ClinicalTrials.gov NCT02613169 (Registered November 24, 2015)

Version: Protocol version 1.7, August 14, 2018.

Keywords: pediatric, tuberculosis, prevention, isoniazid, protocol

ARTICLE SUMMARY

Strengths and limitations of this study

- Most children born to mothers with HIV are HIV-exposed and uninfected (HEU) but remain at high risk of tuberculosis (TB), making them an important population in which to study TB prevention.
- Current TB prevention guidelines do not recommend routine isoniazid preventive therapy (IPT) for children (including HEU) without a known TB contact, though recent data suggest the majority of transmission to children occurs outside the household due community or unperceived household TB, therefore a strength of our study is the enrollment of children without known TB exposure.
- Because of the high risk of progression to TB disease in infants, our strategy focuses on prevention of primary Mtb infection, as detected by a combined endpoint of interferon-gamma release assays (IGRA) and tuberculin skin test (TST) is novel, despite known limitations of both assays.
- Widespread implementation of IPT in adult people living with HIV (including peripartum women) could significantly decrease TB risk in infants, making an HEU-focused TB prevention strategy less needed.
- Given equipoise regarding whether INH prevents Mtb infection in general, and a lack of data specifically among HEU children, a randomized controlled trial design would provide important information regarding INH efficacy for primary prevention in this high-risk population.

INTRODUCTION

HIV-exposed uninfected infants (HEU) in tuberculosis (TB) endemic settings have a high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, even in absence of known Mtb exposure.[1-3] Because infancy is a time of rapid progression from primary infection to active TB[4], it is important to know how TB preventive interventions exert their effects to build new strategies that adapt or extend approaches used in adults. Protecting HEU infants during this vulnerable period of immunodeficiency may provide long term benefits.

Children have a higher rate of progression from Mtb infection to active TB disease than adults.[5, 6] In young children who lack pre-existing adaptive immune responses, Mtb infection progresses rapidly to TB disease, and both innate and early adaptive immune responses may influence susceptibility.[5, 7-9] Virtually all childhood TB disease reflects primary disease, in contrast to adults where a significant portion of disease is due to reactivation of latent TB infection.[10] The World Health Organization (WHO) recommends TB preventive therapy, including isoniazid (INH) isoniazid preventive therapy (IPT) be provided to people living with HIV (PLHIV) >12 months of age to prevent TB.[11] Among children living with HIV (CLHIV), three randomized controlled trials (RCTs) yielded conflicting data regarding whether INH prevents TB disease and/or mortality.[12-15] Only one evaluated HEU infants, and found no protective effect in decreasing a composite outcome of TB disease, Mtb infection (as measured by tuberculin skin test [TST]), or mortality.[12] While previous RCTs have focused on prevention of TB disease, there are scant data regarding INH impact on primary Mtb infection. Among 6-month old HEU infants in Kenya, we found 10% had evidence of Mtb infection by interferon-gamma release assays (IGRAs), suggesting a potential 20% annual cumulative incidence of Mtb infection.[16] In a South African birth cohort including 22% HIV-infected mothers, TST test conversion incidence was 11.8 per 100 child-years, with the majority of conversions occurring before 1 year of age.[17] Among Ugandan children (median age 36 months), prevalence of Mtb infection (either tuberculin skin test (TST) or positive IGRA) was 2-fold higher among

HEU compared to HIV-unexposed children (HUU), with higher prevalence of TST-positivity vs. IGRA in both groups (HEU TST 27.2% vs. IGRA 6.4%, HUU TST 20.6% vs. 1.5%).[18] This suggests HEU infants have a substantial incidence of Mtb infection,[19, 20] as well as low to modest concordance between different Mtb infection measures.

There is currently no gold standard for Mtb infection diagnosis.[21] While TST is recommended in children under 5 years of age,[22, 23] false positivity due to Bacille Calmette-Guérin (BCG) vaccination can occur.[24] Data regarding IGRA performance in young children is scarce, however a recent study of BCG-immunized infants in South Africa indicated high Quantiferon interferon gamma (IFN-γ) conversion values were strongly associated with subsequent development of TB.[25] Because IGRAs offer increased specificity in the presence of recent BCG vaccine, it is plausible they could enhance the ability to measure the preventive effect of INH.[24] Cross-reactivity to non-tuberculosis mycobacteria may cause false positives in both IGRA and TST.[21] IGRA and TST agreement in children varies widely and appears affected by nutrition and HIV status, TB burden, and BCG immunization.[26-30] Recent ATS/IDSA/CDC guidelines recommend dual testing IGRA and TST for groups who are both likely to be infected and at high risk of progression to TB disease as a strategy to increase diagnostic sensitivity.[23] Reduction of specificity with this strategy may be acceptable when consequences of missed Mtb infection (and therefore missed opportunity for treatment) outweigh risks of therapy-associated adverse events. Few longitudinal studies among young infants including HEUs with serial IGRA and TST testing exist.[25, 31] A prospective infant HEU cohort using both IGRA and TST can provide an efficient approach to probe determinants of Mtb infection, more rapidly accruing endpoints (Mtb infection) than studies of TB disease. This study design can contribute unique insights regarding prevention strategies.

Kenyan guidelines mirror WHO guidelines and recommend IPT for all known TB-exposed children <5 years of age and all HIV-infected children >1 year of age regardless of TB-exposure.[11, 32] However for children <5yr without known TB exposure (including HEU), and for HIV-infected children <1 year, IPT is not

recommended.[11, 32] These guidelines illustrate uncertainty regarding IPT in young children, following an RCT from South Africa/Botswana that failed to demonstrate IPT effectiveness in preventing TB disease among CLHIV and HEU <1 year of age without known TB exposure.[12-15] However, it remains possible that among HEU children exposed to unperceived community or household TB, INH may prevent primary Mtb infection. Although data are conflicting, some adult studies have demonstrated IPT benefit in TST- or IGRA-negative adult PLHIV suggesting IPT may confer protection from Mtb infection.[33-36]

The primary goal of this study is to determine whether INH prevents primary Mtb infection in HEU infants, to determine timing and cofactors of primary Mtb acquisition in the first year of life, and to examine the role of immune protective mechanisms in this cohort (**Figure 1**). This paper details the study protocol of an RCT evaluating efficacy of a 12-month course of INH to prevent Mtb infection as measured by IGRA and/or TST in HEU children enrolled at 6 weeks of age in western Kenya.

METHODS AND ANALYSIS

Study Design

The infant TB Infection Prevention Study ("iTIPS") is a 2-arm, non-blinded RCT comparing efficacy of a 12-month course of daily INH vs. no INH to prevent Mtb infection among HEU Kenyan children enrolled at 6 weeks of age (**Figure 2**). Eligible infants are randomized using a 1:1 allocation to INH vs. no INH (**Figure 3**).

Study sites

Kenya is one of 22 high TB burden countries with a generalized TB epidemic[37], with an estimated TB prevalence of 426 per 100,000.[38] This study is conducted in collaborative research sites in western Kenya embedded in Ministry of Health (MOH) maternal child health (MCH) clinics. HIV-infected mothers are followed as part of the national prevention of maternal to child transmission (PMTCT) program and currently receive Option B+ triple antiretroviral therapy.[39] Per Kenyan guidelines, all PLHIV should be screened at routine HIV care visits using symptom-based TB screening and those with negative screens are evaluated for IPT.[11, 37, 39]

Recruitment processes and eligibility criteria

We recruit HIV-infected mothers and their HIV-exposed infants from MCH/PMTCT sites. Infants aged 6 weeks (+4 weeks) of age are eligible for inclusion if they are born to HIV-infected mothers, with birthweight ≥2.5 kilograms, and not born premature (≥37 weeks gestation). Infants with known household TB exposure, including mothers with TB diagnosed in the past year are ineligible. Infants enrolled in other TB prevention or TB vaccine studies are ineligible because these interventions might affect ascertainment of endpoints.

Randomization

Site-stratified randomization is used to allocate infants 1:1 to INH or no INH arms. Randomization numbers were generated prior to study start using STATA 14 "*ralloc*" command with resulting randomization assignment by participant ID printed on cards and placed in opaque envelopes.

Blinding

The study is non-blinded to enable prompt clinical management for any potential drug-related adverse event. IGRAs are performed in the Kenya Medical Research Institute (KEMRI) Centers for Disease Control (CDC) laboratory, which is blinded to arm. The study team administers TST and not blinded to TST result. Data monitoring by the study team is not disaggregated by study arm. The study biostatistician reviews data by arm during preparation of reports to the external Data and Safety Monitoring Board (DSMB). This data is reviewed during closed DSMB sessions, which excludes team members involved in study implementation.

Enrollment and Study procedures

Enrollment

After informed consent is obtained by study staff, household locator information, medical identification number, and cellphone contacts are obtained to facilitate tracing. On enrollment, standardized questionnaires regarding sociodemographic, clinical, obstetric and HIV-related factors, TB exposure and

history, and TB symptoms (for infant, mother, and household members (by maternal report) using WHO symptom screen[40]) are administered (**Supplemental Table 1**). Mothers with suspected TB are referred to the TB program for further screening. If mothers are found to have TB on enrollment, their infants are ineligible for participation and are referred to receive INH per Kenya national guidelines.

Infants undergo physical examination measuring weight, height/length, mid-upper arm circumference, and presence of BCG scar. Medical records are used to abstract data on infant birthweight, PMTCT prophylaxis, other medications, immunizations, and maternal ART regimen, viral load and CD4 cell counts.

Intervention

Isoniazid ~10 mg/kg (7-15 mg/kg) is administered once daily to infants in the INH arm for 12 months. Standardized weight-based dosing (by weight band using 100 mg scored tablets) is used, corresponding to Kenya and WHO recommendations.[32, 41] Pyridoxine is provided to children randomized to INH to decrease peripheral neuropathy risk.[32, 41] Caregivers are advised on how to pulverize isoniazid and pyridoxine to be mixed with small quantities of breastmilk, clean water, or liquid co-trimoxazole to ensure full doses are given and for ease of administration to infants. Participants in the intervention arm are administered daily INH and pyridoxine by caregivers. Infants in the control arm do not receive INH or pyridoxine.

Participant follow-up

Follow-up visits occur at 10 weeks for infants enrolled at 6 weeks of age and at 14 weeks, and 6, 9, and 12 months of age for all participants coinciding with routine Kenya pediatric visit schedule. Follow-up visits include assessment of any TB diagnosis in the mother, infant, or household member since the past visit, as well as any TB symptom in the mother, infant, and household members (by maternal report). Infants in the no INH arm found to have a known TB contact during the study are referred for IPT per Kenyan guidelines. Infants and mothers found to have TB symptoms are referred to the MOH TB Programme for further evaluation. Questionnaires regarding caregiver barriers and facilitators to providing prophylactic

medications (co-trimoxazole, antiretrovirals for PMTCT, and INH [if in INH arm]) are administered at the 6 month of age visit. Endpoint ascertainment occurs at a study visit 12 months post-randomization at approximately 14 months of age.

Sample collection

Infant blood for peripheral blood mononuclear cells (PBMC) and plasma are collected at baseline and visit 2 (10-14 weeks of age). At 12 months post-randomization, blood is collected for IGRA (QFT-Plus) and TST placed and read within 48-92 hours.[42, 43] Infant rectal swabs are collected at enrollment for future gut microbiome studies. Maternal breastmilk and blood for PBMC and plasma separation are collected on enrollment.

Study procedures specific to infants randomized to INH

Liver function tests (LFTs) are performed at enrollment and one month following INH initiation. Adherence is assessed by caregiver report at follow-up visits. Urine is collected at follow-up and study endpoint visits and tested using strips developed to detect INH-metabolites.[44, 45] Hair is collected at study endpoint for future assessment of isoniazid levels as a more objective adherence measure over time.[46, 47]

Safety considerations

IPT has been shown to be safe in prior RCTs and is administered routinely to TB-exposed infants.[12-15] Although routine LFT monitoring is not recommended during INH in children,[22] for this trial baseline LFTs are drawn at enrollment and one month after INH initiation. National Institutes of Health (NIH) Division of AIDS (DAIDS) Table for Grading the Severity of Pediatric Adverse Events is used to grade toxicities.[48] Infants with LFTs $\leq$ grade 2 are allowed to initiate INH. Infants with baseline LFTs $\geq$ grade 3 at baseline have LFTs monitored every 2 weeks and do not initiate INH until LFTs are $\leq$ grade 2. Children are evaluated for peripheral neuropathy using a truncated Denver Developmental test.[49] After INH initiation, if toxicity is suspected, study administered drugs are immediately discontinued and in case of concern for hepatoxicity, LFTs are repeated.

An external and independent DSMB, including experts in pediatric TB, biostatistics, and trial design, monitors severe adverse events (SAE). Summaries of SAEs are given to DSMB members during scheduled meetings. Each SAE is assigned plausibility of relatedness to study drug by study investigators. "Open" reports detailing cumulative overall SAEs are descriptive (no statistical analyses). "Closed" reports of SAEs by study arm are reviewed and the DSMB makes recommendations regarding any imbalances in safety outcomes. O'Brien-Fleming boundaries for benefit and harm are used for interim monitoring and these boundaries are provided by the study statistician in closed reports. The DSMB assesses operational aspects, safety, and effectiveness and makes recommendations regarding study continuation or modifications. Futility is not considered a basis for stopping rules because of the trials' value in understanding correlates of Mtb infection in HEU infants.

Discontinuation, withdrawal, or allocation modification

Participants may withdraw from the study at any point. Study investigators may withdraw a participant on a case-by-case basis if the intervention or study involvement poses a risk to the participant. No modification of allocation will be made. Infants who receive at least one dose of study drug will be included in per protocol analyses. Caregivers of infants who discontinue INH are encouraged to continue study follow-up and endpoint ascertainment.

Data collection and management

Study staff use tablets to collect de-identified data using secure password-protected Research Electronic Data Capture mobile software (REDCap, Vanderbilt University, Nashville, Tennessee, USA).[50] Data are uploaded daily from tablets to the web-based REDCap database. Study investigators will have access to the finalized dataset.

Patient and Public Involvement

Patients were not directly involved in the development of the research question, design of the study, or recruitment. We assess the burden of the trial intervention for participants by gathering data on adverse

events, tolerability of INH, and assessment of caregiver barriers and facilitators to providing prophylactic medications to HEU children via questionnaire. Overall study results will be shared with the clinical facilities and presented to local stakeholders including MOH county and national representatives.

Outcome measures

The primary outcome is Mtb infection by QFT-Plus assay or TST 12 months after enrollment. Similar to QuantiFERON-TB Gold (QFT) IGRA, QFT-Plus measures IFN-γ released by primarily CD4+ T helper lymphocytes after TB-specific antigen (ESAT-6 and CFP-10) stimulation. In addition, QFT-Plus measures IFN-γ released by CD8+ cytotoxic T lymphocytes, after stimulation with the same antigens, which may have increased sensitivity in children, and in populations with lower CD4 counts including HIV.[51, 52] Responses of ≥0.35 IU/ml to TB antigens above the Nil response in either the primarily CD4+ response [TB1], or CD8+ response [TB2] (with Nil <8 IU/ml and a positive mitogen control) are considered positive per manufacture recommendations.[51] A TST of ≥10 mm is considered positive.[22]

Secondary outcomes include severe adverse events (Grade ≥3 per DAIDS Grading Severity of Pediatric Adverse Experiences),[48] use of IFN-γ-independent immune markers in QFT-Plus supernatants to indicate Mtb infection,[53-57] and epidemiologic and immunologic correlates of Mtb infection. Exploratory outcomes include combined endpoint of Mtb infection, TB diagnosis, and/or death, as well as sensitivity analyses of the primary outcome of Mtb infection after excluding infants with evidence of immune responses to ESAT-6 or CFP-10 at enrollment in flow cytometric analyses.

Sample size and power analysis

Assuming alpha of 0.05, power of 0.80, utilizing a 2-sided test, and a 1:1 allocation ratio, with 125 infants in each arm we have power to detect at least 65% decrease in Mtb infection in INH arm vs. control if cumulative incidence of Mtb infection in control arm at 12 months is 0.20, or to detect 70-80% or higher (HR 0.3-0.2) decrease if cumulative incidence of Mtb infection in control arm is 0.15 or 0.10 (**Supplemental Table 2**). To account for loss to follow-up, non-adherence, and isoniazid resistance, we increased sample

size by 20%, with goal enrollment of 300 infants (150 per arm). Baseline characteristics will be compared between randomization arms to assess randomization adequacy.

Statistical methods and analysis

Primary outcome:

Modified Intention-to-treat: We will use a modified intention-to-treat approach, including all participants who underwent randomization irrespective of receiving trial medication with at least one measure of Mtb infection (QFT-Plus or TST), excluding children found to be HIV DNA positive during the study. We will compare the proportion of infants with Mtb infection (either QFT or TST positive) at 12 months between INH and no INH arms using a Chi-squared test and estimate relative risk with 95% confidence intervals using a generalized linear model (GLM) with log link and Poisson family. We will compare cumulative incidence of Mtb infection by arm using a Cox proportional hazard regressions model.

Per protocol: We will evaluate our primary outcome by a per protocol analysis, considering only HEU infants who took at least one dose as taking INH vs. infants who did not take any INH. We anticipate future sensitivity analyses using IPT adherence and continuation data as exposure of interest and Mtb infection as outcome.

Secondary outcome:

Safety and expanded Mtb infection outcomes: For secondary outcomes we will compare proportions of participants by arm using either Chi-squared or Fisher's exact tests as appropriate for $\geq$ grade 3 serious adverse events. In addition, we will conduct secondary analyses using an expanded Mtb infection definition including a positive TST, QFT-Plus, or IFN-γ-independent immune markers in QFT-Plus supernatants.

Epidemiologic and immune correlates of Mtb infection will be assessed using nested case-control studies incorporating all Mtb infections from both arms then conducting stratified analyses in each trial arm to evaluate potential cofactors modified by INH.

Exploratory outcome:

We will compare a composite endpoint of Mtb infection, TB diagnosis, and/or death between randomization groups using a Chi-squared test. Baseline assays may detect evidence of Mtb infection. We will conduct an additional exploratory analysis, incorporating data from baseline assays[58] (utilizing flow cytometry of cryopreserved PBMCs) to exclude infants with evidence of Mtb-specific immune responses to ESAT-6 or CFP-10 at enrollment also using a Chi-squared test.

ETHICS AND DISSEMINATION

Informed consent is obtained from caregivers. The trial protocol is approved by ethical review boards of University of Washington, University of Nairobi/Kenyatta National Hospital, and Jaramogi Oginga Odinga Teaching and Referral Hospital, and Kenya Pharmacy and Poisons Board, and registered at clinicaltrials.gov (NCT02613169). Any protocol changes will be approved by relevant ethical review boards.

The protocol is available at http://depts.washington.edu/gwach/wp-content/uploads/2012/10/iTIPS_Protocol_v1.7_14Aug2018_web.pdf.

We will share trial results at study sites, and with regional and national policymakers. We plan on submitting final results as a peer-reviewed manuscript and will utilize International Committee of Medical Journal Editors authorship criteria. Study investigators will collaborate in writing final study results. HEU children are at increased risk for Mtb infection and TB disease. IPT is not routinely provided to HEU infants in Kenya without evidence of exposure to a known TB case. There is mixed evidence regarding IPT effectiveness to prevent TB disease in infants <1 year. Given potential benefits of IPT to prevent Mtb infection, heightened risk for Mtb infection in this population, and safety of intervention, there is equipoise for randomization.

Trial status

Trial recruitment and enrollment began August 15, 2016. Participant follow-up is anticipated to complete September 2019, with lab analyses anticipated to be completed in December 2019.

DISCUSSION

Isoniazid has proven benefit to treat latent TB infection and prevent active TB disease in HIV-infected and HIV-uninfected populations.[59-63] Data from adult studies in Botswana, South Africa, and Ivory Coast indirectly suggest IPT may prevent Mtb infection; TST negative adult PLHIV who received IPT were protected from active TB, suggesting IPT may both prevent Mtb infection and progression to TB disease.[33-36] IPT has had variable protective efficacy to prevent TB disease and mortality in CLHIV.[12-15] An RCT in South Africa in the pre-ART era randomized CLHIV ≥8 weeks of age to INH vs. placebo independent of reported TB exposure and found INH prevented TB disease by 70% and decreased mortality by 54%, leading to early trial discontinuation.[13] In the observational extension of the trial, combination IPT and ART further decreased TB risk by 11%.[64] However, in a pilot study of CLHIV on ART (median age 35 months) not powered for efficacy, IPT did not exert a significant protective effect on active TB (1.5 vs. 2.9 TB cases per 100 PY, IRR 0.51 [95% CI 0.15-1.75][14]. Similarly, an RCT of INH given for 96 weeks in HIV-infected and HEU infants enrolled at 91-120 days of life in South Africa and Botswana without reported TB exposure, did not prevent TB disease in either group.[12] Furthermore, among HEU, INH did not prevent Mtb infection as measured by a single TST at week 96. In summary, IPT is effective in adults and variably effective for preventing TB disease in HIV-infected and HEU infants, and no trial to date has been designed specifically to evaluate efficacy of IPT to prevent Mtb infection in either adults or children, including both IGRA and TST as an endpoint to both maximize sensitivity of identify Mtb infection.

Study limitations

Enrollment sites are limited to two counties in western Kenya and may not be generalizable to other settings. This area was chosen due to high HIV/TB burden, as well as longstanding collaborations with study investigators in enrolling women and children from MCH/PMTCT clinics.

With non-blinded trials, there are concerns about differential reporting and clinical management. However, one of the composite endpoint components (IGRA status) is assessed in the KEMRI CDC

laboratory, which is blinded to participant INH status. This endpoint is robust and not influenced by unblinded trial design.

We have estimated a substantive INH effect (65% decrease), consistent with TB prevention literature for reduction of TB disease among TST-positive adult PLHIV,[62] but undefined for Mtb infection risk. A larger sample size may be useful if Mtb infection prevalence is lower than anticipated or if INH is less effective in prevention of Mtb infection. We have extended post-trial observational follow-up to 24 months of age to assess longer term Mtb infection incidence. This extended follow-up will allow us to better understand timing of Mtb infection acquisition; however, results will not be included in the trial results because the extended observational period will not include receipt of IPT.

There remains a lack of a gold standard to diagnose Mtb infection;[21] both TST and IGRA are indirect measures of Mtb infection requiring both infection with Mtb and a functioning immune system to mount a positive response. We have incorporated both tests within our composite primary outcome. TST at 12 months may be positive due to BCG exposure at birth rather than Mtb infection. Age at immunization and TST testing timing after BCG administration appears to affect TST reactivity, with younger age at BCG immunization associated with shorter duration of TST reactivity than in adults. In a meta-analysis of 24 studies with >240,000 participants, among participants who were BCG-vaccinated as infants, <1% were TST positive after 10 years post BCG administration, compared to 21% of participants vaccinated after their first birthday who remained TST positive after 10 years post BCG[65]. Similarly, in a recent long-term follow-up study of a BCG vs. placebo trial among Native Americans/Alaskan Natives, BCG administered after 1 year of age was associated with increased incidence of TST reactivity extending up to 55 years after vaccination.[66] Importantly, there is scant data on TST reactivity among BCG-immunized infants TST-tested during first year of life. In a Navajo study in the US, among 250 infants immunized with BCG as newborns, 31% had TST ≥10mm at 3 months which reduced to zero at 9 months of age, suggesting rapid waning of BCG-associated TST responses in children receiving BCG at birth.[67] Therefore, it appears that

TST testing at approximately 1 year of age among children immunized with BCG at birth is more likely to represent Mtb infection, as opposed to BCG-induced reactivity. Non-tuberculosis mycobacteria (NTM) can lead to false positives for both IGRA and TST.[21] Prevalence of NTM disease in Kenya unknown, but in a recent study evaluating 2900 infants for TB incidence in Kenya, 2.6% of infants evaluated for TB had NTMs isolated, though none met American Thoracic Society criteria for NTM disease.[68] Importantly, the study evaluated detection of mycobacteria rather than detection of subclinical NTM infection and there are currently no standard measures for NTM infection. Our study does not include qualitative work to investigate issues of adherence, though does include closed-ended questions regarding caregiver barriers and facilitators to providing prophylactic medications to HEU children.

Kenya endorsed routine IPT for PLHIV in 2014 national guidelines,[39] and counties in which this study is located have had a rapid expansion of IPT as part of routine HIV care. We have described high IPT use in peripartum women.[69] Widespread IPT implementation in adult PLHIV could significantly decrease TB risk in infants, making an HEU-focused TB prevention strategy less needed. Maternal IPT use is not an exclusion criterion. Infant INH drug exposure through breastmilk is very low [70] and unlikely to exert a direct protective effect in the control arm.

Given equipoise regarding whether INH prevents Mtb infection in general, and a lack of data specifically among HEU children, an RCT design could provide important information regarding INH efficacy for primary prevention in this high-risk population.

AUTHOR CONTRIBUTIONS: GJ-S, BAR, JK, SML designed the randomized clinical trial. SML, GJ-S, BAR, TRH, LMC, JK, DM, AW, EM-O developed the study protocol. GJ-S is the principal investigator and protocol chair and TRH is the immunology principal investigator. JK is the protocol co-chair and country principal investigator. EM-O is the Pediatric Clinical TB lead. GJ-S, BAR, SML are responsible for the statistical design of the trial and data analysis of the primary outcomes. SML is the project director and drafted the statistical analysis plan overseen by BAR, the study biostatistician. SML, DM, AW, JK, JNE participated in trial implementation and manuscript preparation. TRH designed the immunologic studies and immunologic work related to the trial. SML wrote the first draft of the manuscript. All authors critically revised, read, and approved the final manuscript.

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TABLES & FIGURE LEGENDS

Figure 1 Study Schema: Aims of RCT to evaluate INH to prevent Mtb infection in HEU infants

Figure 2 Overall Study Strategy

Figure 3 CONSORT diagram

Supplemental Table 1 Overview of study visits and planned procedures

Supplemental Table 2 Primary outcome sample size estimates and power

REFERENCES

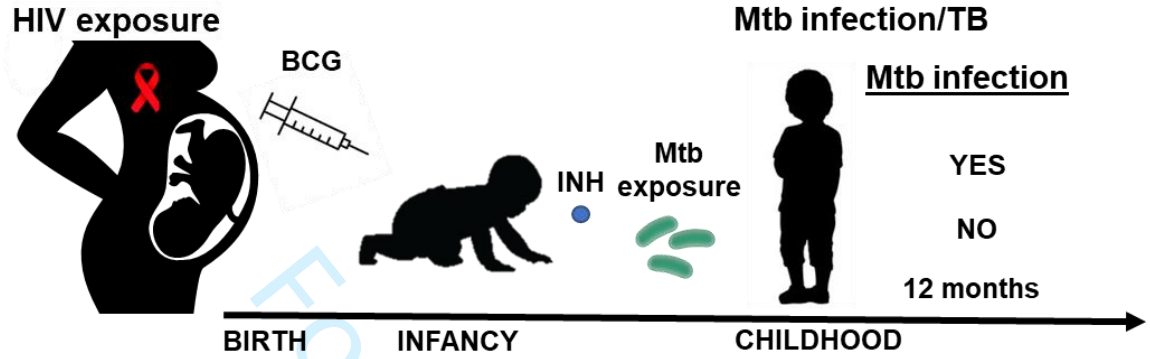
1. Cotton MF, Schaaf HS, Lottering G*, et al.* Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. *Int J Tuberc Lung Dis* 2008,**12**:225-227.
2. Madhi SA, Nachman S, Violari A*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
3. Martinez L, Lo NC, Cords O*, et al.* Paediatric tuberculosis transmission outside the household: challenging historical paradigms to inform future public health strategies. *The Lancet Respiratory Medicine* 2019,**7**:544-552.
4. Marais BJ, Gie RP, Schaaf HS*, et al.* The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004,**8**:392-402.
5. Marais BJ, Gie RP, Schaaf HS*, et al.* The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004,**8**:278-285.
6. Gedde-Dahl T. Tuberculous infection in the light of tuberculin matriculation. *Am J Hyg* 1952,**56**:139-214.
7. Moyo S, Verver S, Mahomed H*, et al.* Age-related tuberculosis incidence and severity in children under 5 years of age in Cape Town, South Africa. *Int J Tuberc Lung Dis* 2010,**14**:149-154.
8. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 1974,**99**:131-138.
9. Starke JR. Childhood tuberculosis during the 1990s. *Pediatr Rev* 1992,**13**:343-353.
10. Donald PR, Maher D, Qazi S. A research agenda to promote the management of childhood tuberculosis within national tuberculosis programmes. *Int J Tuberc Lung Dis* 2007,**11**:370-380.
11. World Health Organization. Latent TB Infection: Updated and consolidated guidelines for programmatic management. 2018. Available at: http://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/. .
12. Madhi SA, Nachman S, Violari A*, et al.* Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011,**365**:21-31.
13. Zar HJ, Cotton MF, Strauss S*, et al.* Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007,**334**:136.
14. Gray DM, Workman LJ, Lombard CJ*, et al.* Isoniazid preventive therapy in HIV-infected children on antiretroviral therapy: a pilot study. *Int J Tuberc Lung Dis* 2014,**18**:322-327.
15. Zunza M, Gray DM, Young T*, et al.* Isoniazid for preventing tuberculosis in HIV-infected children. *Cochrane Database Syst Rev* 2017,**8**:CD006418.
16. Cranmer LM, Kanyugo M, Jonnalagadda SR*, et al.* High prevalence of tuberculosis infection in HIV-1 exposed Kenyan infants. *Pediatr Infect Dis J* 2014,**33**:401-406.
17. Martinez L, le Roux DM, Barnett W*, et al.* Tuberculin skin test conversion and primary progressive tuberculosis disease in the first 5 years of life: a birth cohort study from Cape Town, South Africa. *Lancet Child Adolesc Health* 2018,**2**:46-55.
18. Marquez C, Chamie G, Achan J*, et al.* Tuberculosis Infection in Early Childhood and the Association with HIV-exposure in HIV-uninfected Children in Rural Uganda. *Pediatr Infect Dis J* 2016,**35**:524-529.
19. Bekker A, Du Preez K, Schaaf HS*, et al.* High tuberculosis exposure among neonates in a high tuberculosis and human immunodeficiency virus burden setting. *Int J Tuberc Lung Dis* 2012,**16**:1040-1046.
20. Cotton MF, Schaaf HS, Lottering G*, et al.* Tuberculosis exposure in HIV-exposed infants in a high-prevalence setting. *Int J Tuberc Lung Dis* 2008,**12**:225-227.

21. Pai M, Denkinger CM, Kik SV*, et al.* Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clin Microbiol Rev* 2014,**27**:3-20.
22. American Academy of Pediatrics. Tuberculosis. In: *Red Book 2018-2021 Report of the Committee on Infectious Diseases; American Academy of Pediatrics*. Edited by Kimberlin DW, Brady MT, Jackson MA, Long SA. 31st Edition ed. Elk Grove Village, IL: American Academy of Pediatrics; 2018. pp. 879-853.
23. Lewinsohn DM, Leonard MK, LoBue PA*, et al.* Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. *Clin Infect Dis* 2017,**64**:111-115.
24. Pai M, Behr MA, Dowdy D*, et al.* Tuberculosis. *Nat Rev Dis Primers* 2016,**2**:16076.
25. Andrews JR, Nemes E, Tameris M*, et al.* Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study. *The Lancet Respiratory Medicine* 2017,**5**:282-290.
26. Velasco-Arnaiz E, Soriano-Arandes A, Latorre I*, et al.* Performance of Tuberculin Skin Tests and Interferon-gamma Release Assays in Children Younger Than 5 Years. *Pediatr Infect Dis J* 2018,**37**:1235-1241.
27. Mandalakas AM, van Wyk S, Kirchner HL*, et al.* Detecting tuberculosis infection in HIV-infected children: a study of diagnostic accuracy, confounding and interaction. *Pediatr Infect Dis J* 2013,**32**:e111-118.
28. Verhagen LM, Maes M, Villalba JA*, et al.* Agreement between QuantiFERON(R)-TB Gold In-Tube and the tuberculin skin test and predictors of positive test results in Warao Amerindian pediatric tuberculosis contacts. *BMC Infect Dis* 2014,**14**:383.
29. Debulpaep S, Corbiere V, Levy J*, et al.* Contribution of QuantiFERON-TB Gold-in-Tube to the Diagnosis of Mycobacterium tuberculosis Infection in Young Children in a Low TB Prevalence Country. *Front Pediatr* 2019,**7**:291.
30. Elliot C, Marais B, Williams P*, et al.* Tuberculin skin test versus interferon-gamma release assay in refugee children: A retrospective cohort study. *J Paediatr Child Health* 2018,**54**:834-839.
31. Cranmer LM, Draper HR, Mandalakas AM*, et al.* High Incidence of Tuberculosis Infection in HIV-exposed Children Exiting an Isoniazid Preventive Therapy Trial. *Pediatr Infect Dis J* 2018,**37**:e254-e256.
32. Kenya Ministry of Health. National Guidelines on Management of Tuberculosis in Children, Third Edition. 2017. Available at: https://www.chskenya.org/wp-content/uploads/2018/03/National-Guidelines-on-Management-of-Tuberculosis-in-Children.pdf.
33. Samandari T, Agizew TB, Nyirenda S*, et al.* 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011,**377**:1588-1598.
34. Rangaka MX, Wilkinson RJ, Boulle A*, et al.* Isoniazid plus antiretroviral therapy to prevent tuberculosis: a randomised double-blind, placebo-controlled trial. *Lancet* 2014,**384**:682-690.
35. Temprano ANRS Study Group, Danel C, Moh R*, et al.* A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa. *N Engl J Med* 2015,**373**:808-822.
36. Badje A, Moh R, Gabillard D*, et al.* Effect of isoniazid preventive therapy on risk of death in west African, HIV-infected adults with high CD4 cell counts: long-term follow-up of the Temprano ANRS 12136 trial. *Lancet Glob Health* 2017,**5**:e1080-e1089.
37. World Health Organization. Global tuberculosis report 2018. 2018. Available at: http://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf?ua=1.
38. Kenya Ministry of Health. Kenya Tuberculosis Survey 2017. Available at: https://www.nltp.co.ke/survey-reports-2/.

39. Kenyan Ministry of Health, National AIDS and STI Control Program (NASCOP). Guidelines on use of antiretroviral drugs for treating and preventing HIV infection: a rapid advice. 2014. Available at: http://healthservices.uonbi.ac.ke/sites/default/files/centraladmin/healthservices/Rapid%20Advice%20Booklet%202014%2024%20June%2012%20noon_0.pdf.
40. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. 2011. Available at: http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf.
41. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children, 2nd edition. 2014. Available at: http://www.who.int/tb/publications/childtb_guidelines/en/.
42. Cobelens F, Van Deutekom H, Draayer-Jansen I*, et al.* Tuberculin skin test reactions by time of reading among Dutch travellers. *Int J Tuberc Lung Dis* 2003,**7**:758-763.
43. Tuberculin reaction size on five consecutive days. *Bull World Health Organ* 1955,**12**:189-196.
44. Nackers F, Huerga H, Espie E*, et al.* Adherence to self-administered tuberculosis treatment in a high HIV-prevalence setting: a cross-sectional survey in Homa Bay, Kenya. *PLoS One* 2012,**7**:e32140.
45. Meissner PE, Musoke P, Okwera A*, et al.* The value of urine testing for verifying adherence to anti-tuberculosis chemotherapy in children and adults in Uganda. *Int J Tuberc Lung Dis* 2002,**6**:903-908.
46. Gerona R, Wen A, Chin AT*, et al.* Quantifying Isoniazid Levels in Small Hair Samples: A Novel Method for Assessing Adherence during the Treatment of Latent and Active Tuberculosis. *PLoS One* 2016,**11**:e0155887.
47. Mave V, Chandanwale A, Kinikar A*, et al.* Isoniazid hair concentrations in children with tuberculosis: a proof of concept study. *Int J Tuberc Lung Dis* 2016,**20**:844-847.
48. Division of AIDS National Institute of Allergy and Infectious Diseases. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. 2014. Available at: http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf.
49. IMPAACT P1041 trial protocol. 2007. Available at: https://impaactnetwork.org/studies/P1041.asp.
50. Harris PA, Taylor R, Thielke R*, et al.* Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009,**42**:377-381.
51. Qiagen. QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA Package Insert. 2014. Available at: http://www.quantiferon.com/irm/content/PI/QFT/PLUS/2PK-Elisa/UK.pdf.
52. Rozot V, Patrizia A, Vigano S*, et al.* Combined use of Mycobacterium tuberculosis-specific CD4 and CD8 T-cell responses is a powerful diagnostic tool of active tuberculosis. *Clin Infect Dis* 2015,**60**:432-437.
53. Armand M, Chhor V, de Lauzanne A*, et al.* Cytokine responses to quantiferon peptides in pediatric tuberculosis: a pilot study. *J Infect* 2014,**68**:62-70.
54. Kim SY, Park MS, Kim YS*, et al.* The responses of multiple cytokines following incubation of whole blood from TB patients, latently infected individuals and controls with the TB antigens ESAT-6, CFP-10 and TB7.7. *Scand J Immunol* 2012,**76**:580-586.
55. Lighter-Fisher J, Peng CH, Tse DB. Cytokine responses to QuantiFERON(R) peptides, purified protein derivative and recombinant ESAT-6 in children with tuberculosis. *Int J Tuberc Lung Dis* 2010,**14**:1548-1555.
56. Rubbo PA, Nagot N, Le Moing V*, et al.* Multicytokine detection improves latent tuberculosis diagnosis in health care workers. *J Clin Microbiol* 2012,**50**:1711-1717.

57. Biselli R, Mariotti S, Sargentini V*, et al.* Detection of interleukin-2 in addition to interferon-gamma discriminates active tuberculosis patients, latently infected individuals, and controls. *Clin Microbiol Infect* 2010,**16**:1282-1284.
58. Warr AJ, Shah JA, LaCourse SM*, et al.* Mycobacterium tuberculosis antigen-specific T-cell responses in HIV-infected mothers and their infants. In: *Keystone Symposia on Tuberculosis: Translating Scientific Findings for Clinical and Public Health Impact*. Whistler, British Columbia, Canada; April 15-19, 2018.
59. Comstock GW, Ferebee SH, Hammes LM. A controlled trial of community-wide isoniazid prophylaxis in Alaska. *Am Rev Respir Dis* 1967,**95**:935-943.
60. Comstock GW, Baum C, Snider DE, Jr. Isoniazid prophylaxis among Alaskan Eskimos: a final report of the bethel isoniazid studies. *Am Rev Respir Dis* 1979,**119**:827-830.
61. Smieja MJ, Marchetti CA, Cook DJ*, et al.* Isoniazid for preventing tuberculosis in non-HIV infected persons. *Cochrane Database Syst Rev* 2000:CD001363.
62. Akolo C, Adetifa I, Shepperd S*, et al.* Treatment of latent tuberculosis infection in HIV infected persons. *The Cochrane database of systematic reviews* 2010:CD000171.
63. Ayele HT, Mourik MS, Debray TP*, et al.* Isoniazid Prophylactic Therapy for the Prevention of Tuberculosis in HIV Infected Adults: A Systematic Review and Meta-Analysis of Randomized Trials. *PLoS One* 2015,**10**:e0142290.
64. Frigati LJ, Kranzer K, Cotton MF*, et al.* The impact of isoniazid preventive therapy and antiretroviral therapy on tuberculosis in children infected with HIV in a high tuberculosis incidence setting. *Thorax* 2011,**66**:496-501.
65. Farhat M, Greenaway C, Pai M*, et al.* False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006,**10**:1192-1204.
66. Mancuso JD, Mody RM, Olsen CH*, et al.* The Long-term Effect of Bacille Calmette-Guerin Vaccination on Tuberculin Skin Testing: A 55-Year Follow-Up Study. *Chest* 2017,**152**:282-294.
67. Lifschitz M. The value of the tuberculin skin test as a screening test for tuberculosis among BCG-vaccinated children. *Pediatrics* 1965,**36**:624-627.
68. Kaguthi G, Nduba V, Murithi W*, et al.* The Incidence of Non-Tuberculous Mycobacteria in Infants in Kenya. *J Trop Med* 2019,**2019**:1273235.
69. LaCourse SM, Wagner AD, Cranmer LM*, et al.* Brief Report: High Programmatic Isoniazid Preventive Therapy (IPT) Use in Pregnancy Among HIV-Infected Women. *J Acquir Immune Defic Syndr* 2019,**82**:41-45.
70. Garessus EDG, Mielke H, Gundert-Remy U. Exposure of Infants to Isoniazid via Breast Milk After Maternal Drug Intake of Recommended Doses Is Clinically Insignificant Irrespective of Metaboliser Status. A Physiologically-Based Pharmacokinetic (PBPK) Modelling Approach to Estimate Drug Exposure of Infants via Breast-Feeding. *Front Pharmacol* 2019,**10**:5.

Figure 1: Aims of RCT to evaluate INH to prevent Mtb infection in HEU infants



DOES INH PREVENT PRIMARY MTB INFECTION in HEU?

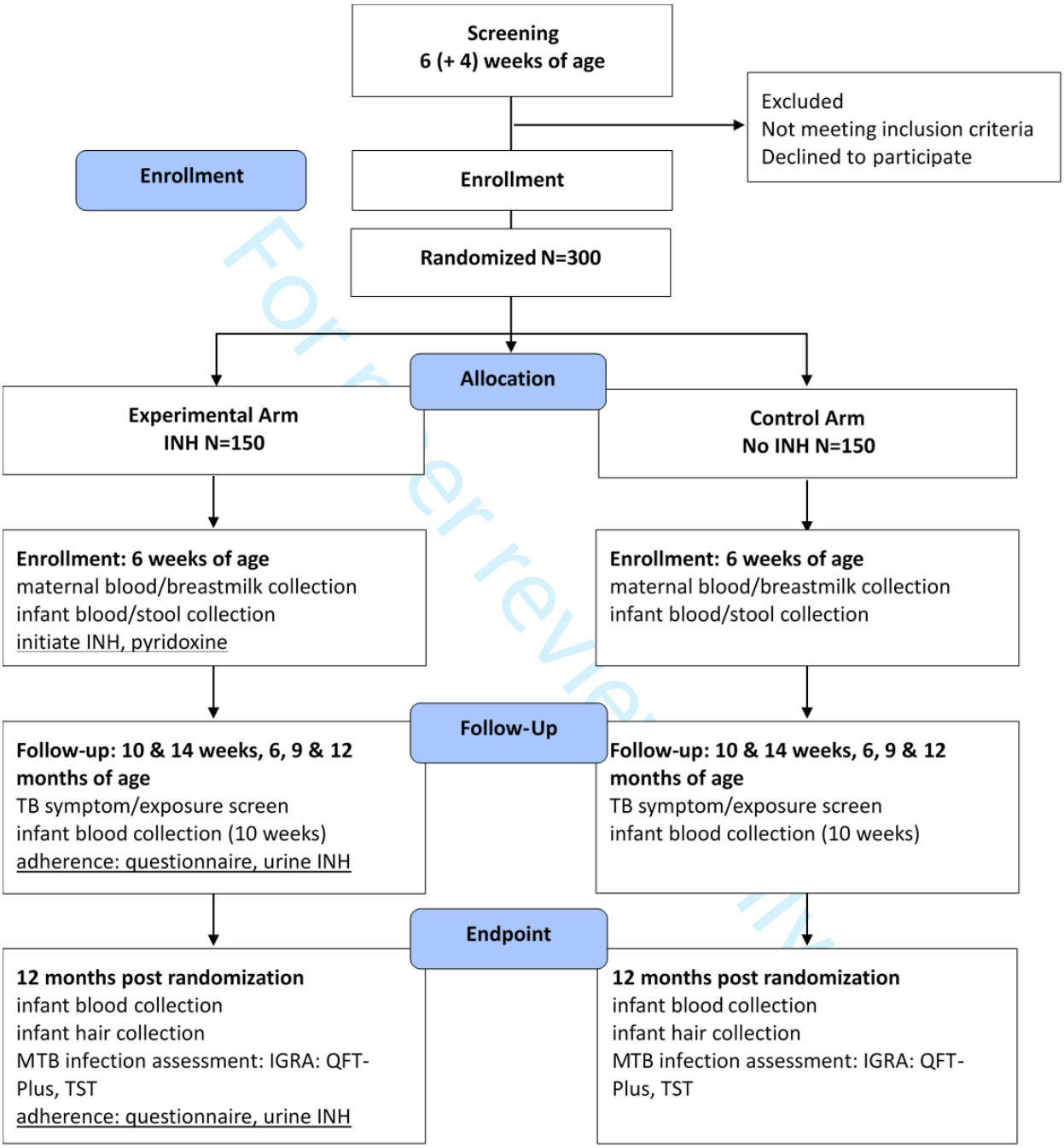
Epidemiologic (**AIM 2**) and immunologic (**AIM 3**) correlates of Mtb infection?

- Infant BCG-specific T-cell responses (blood)
- Maternal Mtb-specific T-cell responses (blood/breastmilk)
- Does INH modify these responses?

Figure 2: Overall Study Strategy

Study Design	Non-blinded randomized controlled trial
Intervention	Intervention: INH for 12 months Control group: No INH
Primary Outcomes	Aim 1: Mtb infection in HEU infants at 12 months post enrollment as measured by IGRA (QFT-Plus) and/or TST Aim 2: Epidemiologic correlates of infant Mtb infection Aim 3: Immunologic correlates of infant Mtb infection
Population	HEU infants ~6 weeks of age and their HIV-infected mothers
Exclusions	• Infants with known exposure to active TB in household • Positive HIV DNA at 6 weeks • Premature and/or birthweight 2.5 kg
Target enrollment	300 HEU infants and their HIV-infected mothers (150 each arm)
Sampling framework	Consecutive enrollment of HEU infants and their HIV-infected mothers at MCH/PMTCT clinics in western Kenya

Figure 3: CONSORT diagram



Supplemental Table 1: Overview of study visits and planned procedures				
	Enrollment 6 weeks of age	**Follow-up visit** [a]	**Endpoint 12 months post enrollment**	**TB Diagnosis**
HIV testing (per MOH)	**x**	**x**[b]	**x**	
Enrollment	**x**			
Sociodemographic survey	**x**	**x**	**x**	
Health history	**x**	**x**	**x**	
Physical exam	**x**	**x**	**x**	
TB symptom screen	**x**	**x**	**x**	
SAE assessment		**x**	**x**	
Adherence assessment via questionnaire, urine INH testing[c]		**x**	**x**	
TB exposure assessment	**x**	**x**	**x**	
Infant blood draw (PBMC/plasma, LFT[c])	**x**[d]	**x**[d]		
Maternal blood draw (PBMC/plasma)	**x**			
Maternal breastmilk collection	**x**			
Infant stool collection	**x**			
Infant TST placement			**x**[e]	**x**[e]
Infant blood draw (IGRA)			**x**[e]	**x**[e]
Infant hair collection [c]			**x**	

[a] Follow up visits will occur at 10 and 14 weeks of age, and 6, 9, and 12 months of age.

[b] Infant DNA PCR will be drawn at 6 weeks of age and HIV antibody test will be drawn at 12 months of age per Kenyan MOH guidelines.

[c] For infants randomized to INH

[d] For all infants blood will be drawn for PBMCs and plasma at the 10 week of age visit. For infants randomized to INH arm, LFTs will be drawn at baseline (6 weeks) and 10 weeks of age (1 month post INH initiation).

[e] Blood will be drawn to assess the presence of Mtb infection at study endpoint, time of TB diagnosis, and in the event of study withdrawal using QFT-plus. If blood volume is insufficient for QFT-Plus (<4ml), blood will be processed for PBMCs for flow cytometry-based assessment of Mtb infection. TST will be placed and read within 48-96 hours.

Supplemental Table 2: Primary outcome sample size estimates and power			
Power 80%, 2-sided p 0.05 1 year follow-up	Maximum HR for IPT detectable	Risk of Mtb infection after 12 months	Number per arm
0.2 risk Mtb infection after 12 months follow-up	0.5	0.2	220
	0.4	0.2	150
	0.35	0.2	120
	0.32	0.2	100
	0.2	0.2	55
0.15 risk Mtb infection after 12 months follow-up	0.5	0.15	300
	0.4	0.15	180
	0.35	0.15	150
	0.31	0.15	120
	0.2	0.15	75
0.10 risk Mtb infection after 12 months follow-up	0.5	0.1	420
	0.4	0.1	270
	0.35	0.1	220
	0.3	0.1	180
	0.2	0.1	110

Gray shaded – Expected study target

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	0
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	confirmed
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	0, 16
	5b	Name and contact information for the trial sponsor	0
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	9
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	12

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Appendix 1: Consent
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Appendix 1: Consent
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	16
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1: Consent
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Appendix 1: Consent

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

UNIVERSITY OF WASHINGTON (UW) and UNIVERSITY of NAIROBI (UoN) Collaborative Study Group

CONSENT and PARENTAL PERMISSION FORM FOR RANDOMIZED TRIAL

Preventing *Mycobacterium tuberculosis* Infection in HIV-Exposed Infants

Short Title: Infant TB Infection Prevention Study ("iTIPS")

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1. **Researcher's Statement**:

We are asking you and your child to be in a research study. The purpose of this form is to give you the information you will need to help you decide whether you and your child will be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want to be in the study or not. This process is called "informed consent." This form serves as a both as a record of your consent to be in the study and as a parental permission form. We will give you a copy of this form for your records.

The word "you" in this form refers to you and your child.

2. **What you should know about this study:**

- This form explains what would happen if you join this research study.
- Please read it carefully. Take as much time as you need.
- Please ask the research team questions about anything that is not clear.
- You can ask questions about the study at any time.
- If you choose not to be in this study, it will not affect any other care received at clinic.
- If you say 'Yes' now, you can still change your mind later.
- You can quit the study at any time.
- You would not lose benefits or be penalized if you decide not to take part in the study or to quit the study later.

3. **What is the goal of this study?**

The goal of any research study is to answer questions. We (the research team listed on the front of this form and our staff) are doing this research study to answer the following question:

- Does the medicine isoniazid (INH) decrease the risk of *Mycobacterium tuberculosis* (MTB) infection?

MTB causes the disease called tuberculosis (TB). Once infected with MTB, some people go on to get TB disease. Young children, as well as people with HIV are more likely to get TB disease because their body defenses are sometimes weak. Children, even if they are not infected with HIV, are more likely to become infected with TB if someone else in their household is also infected with both HIV and TB. INH has been

successfully used to decrease the chances of getting TB disease after MTB infection, but little is known whether it can prevent getting MTB infection in the first place.

4. **Why do I have the option of joining this study?**

You have the option to take part in this research study by being an HIV-infected mother and having a baby that was exposed to HIV.

5. **How many people will take part in this study?**

We think that about 300 mothers and their infants will take place in this research study at sites in western Kenya.

6. **If I agree to join this study, what would I need to do?**

STUDY PROCEDURES

This is what will happen if you agree to participate in this study. We will ask you to read, discuss, and sign or make your mark on this form. After this form is signed or marked, the study staff will ask you questions about you and your child's health, including HIV status, questions about your pregnancy, medications, and if you have been exposed to someone with TB. A study clinician will collect up to 5 mls of blood (teaspoon) from your infant on enrollment at 6 (+4) weeks of age, 10 weeks (+/- 4) weeks of age and 12 months after starting the study, and if your infant is diagnosed with TB disease during the study. We will also ask for a stool sample from your infant. You will also be asked to give 30 mls of breast milk and 5 mls of blood at the beginning of the study. These samples will be used to study the body's defenses against TB.

You will be placed into 1 of 2 groups. You cannot choose which group you will be placed in. You will be placed in a group by chance based on a number that has been assigned to you. The two groups you may be assigned to include either 1) INH or 2) no INH. Your child's chance of getting INH or no INH is the same, just like flipping a coin.

INH group - If your infant is assigned to the INH group, you will be asked to give your child INH daily for 1 year. Although INH is well tolerated in infants, it can be associated in very rare cases with tingling, burning, or numbness in the hands and feet. To prevent this, you will also be asked to give you child a vitamin called pyroxidine. At the beginning of the study, you will be asked how you would like to receive your child's medication every month. You can choose to come to the clinic to pick it up or have a field worker bring it to your home. Also, if your child is in the INH group we will draw blood to measure your infant's baseline liver function at enrollment (before staring INH) and after taking the medicine for 4-6 weeks. The liver is the main part of the body that filters this medication in the body.

No INH group – If your infant is assigned to the no INH group, you will still have the same study procedures performed as the INH group, such as exams and blood draws, except your child will not be given INH or pyroxidine.

These tests and exams help us find out if being in this study causes any effects that are important to know about. We use them to check on the safety of the people in this study. We also use them to learn if the experimental treatment is helping or not.

You will be asked to bring your infant to clinic to be evaluated on enrollment at approximately **6 weeks of age (enrollment), 10 weeks, 14 weeks, 6 months, 9 months, and 12 months of age**. These visits are aligned with the Kenyan recommended schedule of pediatric well child/immunization visits. Additionally, you will be asked to come to the last study visit at **12 months post-enrollment**. It is very important to come to the last visit as this is the visit when we will draw blood to see if your infant has been infected with tuberculosis. If your infant is in the INH group, you will be given enough INH and pyroxidine at each visit to last until the next visit.

Tuberculin skin test (TST). A study clinician will a small needle to put some testing material, called tuberculin, just under the skin of your infant at the end of study visit and if your infant is diagnosed with TB. We will ask you to return to the clinic in 2-3 days to check the result by measuring if there is a reaction on your skin. TST is a test that is used to diagnose MTB infection, but does not necessarily mean you have TB disease.

These tests and exams help us find out if being in this study causes any effects that are important to know about. We use them to check on the safety of the people in this study. We also use them to learn if the experimental treatment is helping or not.

Blood for genetic testing – Some of the blood drawn at the beginning of the study from your infant will be stored to do a test to check the genes (NAT2) that are related to how the body filters INH. The samples that will be tested will be chosen after the study is completed. You will not be told of the result for this test because it is for investigation only and will be done after the study is completed.

Urine and hair for INH testing – For children in the INH group, we will collect urine at the 10 weeks, 14 weeks, 6 months, 9 months, 12 months of age, and study endpoint visit (approximately 14 months of age). This urine will be used to test for INH in the clinic using a dipstick. We will also cut a small thatch of hair (approximately 30 strands) at the end of study visit. This hair will be used to measure INH. You will not be told of the result for this test on hair because it is for investigation only and will be done after the study is completed.

MEDICAL RECORD INFORMATION

We will ask for access to your and your baby's clinic and pharmacy records to find out more information about your pregnancy, delivery, and postpartum care. If you agree to give us access to your medical records, we will get information from the clinics where you received care before, during, and after delivery of your baby, including: any health problems, medication adherence and side effects, and your baby's health information. We will also record laboratory test results, like your CD4 and HIV viral load tests, and infant's HIV tests.

7. **How long would I be in this study?**

If you choose to take part in all the study visits, you and your infant would be in the study for 1 year. If you join this study, you can decide to stop **at any time, for any reason**. If you decide to stop you would need to talk with site investigators so you leave the study in a safe way.

The research study clinicians could also decide to take you out of this study. This might happen if we find out that it is not safe for you to continue in the study. It may also happen if you cannot come to enough of the study visits. If we ask you to leave the study we would always explain why and this would not hamper other care received at the facility in any way.

8. **What are the potential harms or risks if I join this study?**

There are potential harms or risks if you take part in this study. Some are common and some are rare. They are described below.

Potential Harms and Discomforts (from the most common, to the most rare):

- Local irritation due to blood draw
- Local irritation due to TST
- Maternal breast discomfort due to self-expression of breast milk
- Nausea, vomiting, stomach discomfort due to INH
- Peripheral neuropathy (numbness, tingling of the nerves in your hands and feet) due to INH
- Hepatitis (irritation of the liver which is the organ that filters the medicine INH)
- Some people feel uncomfortable answering questions about their health and their baby's health

Because this research study involves a medication that has been used primarily to treat TB disease or prevent TB disease in the past (not prevent MTB infection): we do know that in general INH is generally well tolerated by infants.

A Data Safety Monitoring Board (DSMB) will review the information from this research study. This board is made up of a group of experts responsible for looking at how people in the research study are doing. If you take part, we would tell you about any new information we learn that might affect your health or your willingness to stay in the study.

9. **What are the potential benefits if I join this study?**

Potential Benefits for You:

Being in this study might benefit you in the following ways:

- Participants will benefit from direct medical care in the long-term research group.

Potential Benefits for Others:

- All infants - those born to HIV infected mothers and those born to uninfected mothers - will benefit from a better understanding in how the body defenses protect against MTB infection
- We hope to use information we gain in this study to benefit others in regions with high tuberculosis rates.

10. **What other options do I have?**

Whether or not you decide to participate in this research study, you can continue to receive your mother-child health care at this clinic.

11. **Who is funding this study?**

The study team and/or the University of Washington and Kenyatta National Hospital are receiving financial support from the Thrasher Research Foundation Health in the United States.

12. **How would you keep my information confidential?**

We will keep your identity as a research subject confidential. Your HIV test results, your infants MTB infection test results, medical records, and responses to questions will be kept private, and no identifying information of any kind will be released to any other person or agency that is not working on this study, without your permission in writing. We will not publish or discuss in public anything that could identify you. Any specimens you provide, and your medical information will be identified by a code number. All of your information, including the link between your name and code number will be kept in a secure location at the clinic only. Once the study is completed, we will maintain the link for 5 years, after this time we will remove your name and all identifying information from the study files. Any publication of this study will not use your name or identify you personally. However, study team may share identifiable information about you in the case the study team becomes aware of possible harm to yourself or others.

Although we will make every effort to keep your information confidential, no system for protecting your confidentiality can be completely secure. It is still possible that someone could find out you were in this study and could find out information about you. Government or university staff may review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

Study records may be reviewed by:

- University of Washington, including the Institutional Review Board
- Kenyatta National Hospital and University of Nairobi, including the Ethics and Research
- Committee
- Kenya Medical Research Institute (KEMRI)

A description of this clinical trial will be available on http://www.clinical.trials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time. A copy of your consent form will be placed in your study record.

13. **Would it cost me money to be in this study?**

If you take part in this study there would be no cost to you.

14. **What if I were injured because I joined this study?**

If you think you or your infant has a medical problem or illness related to this research, contact Dr. John Kinuthia**:** +254-722-799-052 right away. He will treat you or refer you for treatment. If your child is injured as a result of being in the study, you will be offered free care at the study clinic. If you require medical care that the study clinic cannot provide, we will refer you to the appropriate organizations to receive care for the injury. The costs of the treatment may be billed to you or the National Hospital Insurance Fund (NHIF) just like other medical costs, or it may be covered by the UW's discretionary Human Subject's Assistance Program (HSAP) depending on a number of factors. The researcher may request HSAP coverage by following established procedures. If you wish to request HSAP coverage yourself, you may contact the

researchers listed on the first page, or the UW Human Subjects Division at hsdinfo@uw.edu or +1-206-543-0098. Ask the researchers if you would like information about the limits and conditions of the HSAP. The UW does not normally provide any other form of compensation for Injury. However, the law may allow you to seek payment for injury-related expenses if they are caused by malpractice or the fault of the researchers. You do not give up any legal rights by signing this consent form.

15. **Would I be paid of I join this study?**

Participants will be provided a stipend for travel.

16. **If I join the study, can I stop?**

Yes. Taking part in research is always a choice. If you decide not to be in the study, you can change your mind at any time. We ask that you tell Daniel Matemo who can be reached at +254-722-322-378.

If you choose to leave the study, it will not affect your care at the study site. You will not lose any benefits or be penalized if you choose to leave the study. We may ask you to come for a visit if you leave the study early. At that visit we may ask to collect 5 mls of blood (teaspoon) and place a TST like we would at the end of the study visit.

17. **Will my samples be used after this study is done?**

We would like to save samples of your blood and breast milk and your baby's blood,stool, and hair at the KEMRI/CDC, University of Nairobi, the University of Washington, the Fred Hutchinson Cancer Research Center, Emory University, or the University of California, San Francisco, for future HIV and/or TB related research and maternal and infant health. This may include testing for genes which may affect whether a person is more or less likely to get infections, or things that may affect infant and maternal health (mother's health during postpartum period with special emphasis on HIV-related illnesses, infant health with special emphasis on HIV-exposure, and TB exposure).

Information we get from you, and your samples, may be shared with other investigators studying HIV, TB, or mother and child health. We will not share your name or any identifying information with them. An Institutional Review Board or Independent Ethics Committee, which looks at study application to ensure the safety and rights of research participants, must approve future research studies in which we will use your or your baby's samples to obtain information about both of you. Permission from the University of Nairobi's Ethics Committee will be sought before any of these samples are used for future research. These tests are for research and are not useful for your or your baby's clinical care. Before your samples or your baby's samples leave the clinic, they will be assigned a code and your name or your baby's name will not be on them. We will store these samples for ten years after completion of the study. Storage of samples past this time period will only occur with approval from an Institutional Review Board and Ethics Committee.

If you do not want to have your or your baby's samples saved for future research, you can still be in this study and your or your baby's samples will be destroyed once testing for the study is completed. If you agree to store your or your baby's samples now, but change your mind before the end of the study, let the study staff know and we will make sure that your or your baby's samples do not get stored for future research. We will not sell your or your baby's samples. Tests done on your or your baby's samples may lead to a new invention or discovery. We have no plans to share any money or other benefits resulting from any potential invention or discovery with you.

CONSENT FOR STUDY PARTICIPATION

Subject's statement:

This study has been explained to me. I volunteer to take part in this research. I have had a chance to ask questions. If I have questions later about the research, I can ask one of the researchers listed above. If I have questions about my rights as a research subject, I can call the *Kenyatta National Hospital Ethics and Research Committee, at 2726300 Ext. 44102*. I give permission to the researchers to use my medical records including my baby's as described in this consent form. I will receive a copy of this consent form.

Printed name of subject	Signature or thumbprint of subject	Date
Witness Name (if caregiver illiterate)	Witness Signature	Date

CONSENT FOR SAMPLE STORAGE FOR FUTURE STUDIES INCLUDING GENETIC TESTING

Please mark, one option for each question below:

YES ___NO___ You can store **my** samples for **future research** into HIV, TB or maternal child health

YES ___NO___ You can store samples from **my baby** for **future research** into HIV, TB or maternal child health

YES ___NO___ You can store my samples for future research into HIV, TB or maternal child health **including genetic testing**

YES ___NO___ You can store samples from my baby for future research into HIV, TB or maternal child health **including genetic testing**

Printed name of subject	Signature or thumbprint of subject	Date
Witness Name (if caregiver illiterate)	Witness Signature	Date

Who do I contact if I have problems or questions?

If you ever have any questions about this study, or if you have a research-related injury, you should contact Dr. John Kinuthia. If you have questions about your rights as a research participant, you should contact the Kenyatta National Hospital Ethics and Research Committee, at 2726300 Ext. 44102.

Printed name of study staff obtaining consent	Signature	Date

Copies to: Researcher, Participant